THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Committee of Management

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A. T. R. MATTICK, B.Sc., Ph.D.
The National Institute for Research in Dairying, University of Reading

and

N. C. WRIGHT, M.A., D.Sc., Ph.D.
The Hannah Dairy Research Institute, Kirkhill, Ayr

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VOLUME 13

CAMBRIDGE
AT THE UNIVERSITY PRESS
1944

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Cambridge University Press
LONDON: BENTLEY HOUSE

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PRINTED IN GREAT BRITAIN

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J821
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292. THE EFFECT OF A VITAMIN A-RICH DIET ON THE VITAMIN A CONTENT OF THE COLOSTRUM OF DAIRY COWS

BY J. STEWART AND J. W. McCALLUM, *Moredun Institute, Gilmerton, Midlothian*

In previous papers by the authors(1,2) it was shown that the vitamin A content of the colostrum of cows varied within wide limits and that the wide range was not due to any differences in dietary, breed or date of calving in animals kept under the same conditions of management. It was also established that calves born of cows whose colostrum possessed a vitamin A content of less than 250 blue units (Moore's) per 100 ml. were more liable to infections such as white scour, navel-ill and joint-ill than were calves from cows whose colostrum had a vitamin A content of more than 250 blue units per 100 ml.

In the light of the above findings it was considered that the occurrence of white scour and allied infections might be considerably lessened in herds if the vitamin A content of the colostrum of all the cows in the herd was maintained at a high level so that each calf would receive at birth a sufficiency of vitamin A.

Since it is commonly accepted that the vitamin A content of the colostrum depends directly on the liver reserves of this vitamin it was considered that the best method to ensure a high vitamin A content of the colostrum would be to keep the liver reserves as high as possible at parturition. During 1938–9 an experiment was carried out based on the above assumption. A foodstuff high in carotene was sought—a foodstuff which would be eaten readily by dairy cows, which could be added to a dairy ration without interfering with the nutritive ratio and which could be grown by the dairy farmer if it became an integral part of his dairy ration. Carrot was chosen as being highest in carotene.

Five farms were selected, of which the owners agreed to supplement the diet of a number of their cows with carrots and to keep as controls others which were fed the same ration with the exception of carrots. In all, sixty-nine cows were fed carrots and sixty-seven acted as controls. In order to keep the liver reserves as high as possible the carrots were fed from the day the cows came off pasture in October till they calved during the months from November to April. The carrots were fed at the rate of 3 lb. per animal per day. This, even when the cows were lactating, was considered ample to provide all the vitamin A being excreted in the milk, and to ensure during the dry period very high body reserves being accumulated before parturition.

The animals, once they became accustomed to the diet, ate the carrots avidly. The controls were so chosen that each one would calve as near as possible to the date on which a carrot-fed cow was due to calve. This ensured that the calving dates of the carrot-fed cows and controls were equally spaced out over the calving season and that the length of the period on stall rations before parturition would be a constant factor between the two groups. A colostrum sample was obtained within 1 hr. of calving and despatched to the laboratory where, on arrival within 24 hr., the vitamin A content was estimated according to the methods in use at this laboratory(1). As a check a second colostrum sample was taken 24 hr. after the first.

In order to save space the results have been tabulated as shown in Table 1. The number of cows in both the control and carrot-fed groups are given for each 100 units from 0 to 2500. The numbers are recorded for both blue, yellow and international units.

If the columns of blue units (Moore's) are examined there is no marked variation observed between the distribution of the members of the control group and those of the carrot-fed group. In both groups the range for the vitamin A content of the colostrum is practically identical, with the tendency for more cows of the control group to be above the 1000 blue units mark as compared with those of the carrot-fed group. It will be noted that the cow secreting the colostrum with the highest number of blue units per 100 ml. belonged to the control group.

Table 1. *Showing distribution of control and carrot-fed cows according to content of vitamin* A *for* 100 *ml. of their colostrum*

	Blue units (Moore's)		Yellow units (Moore's)		International units	
Units per 100 ml.	No. of control animals	No. of carrot-fed animals	No. of control animals	No. of carrot-fed animals	No. of control animals	No. of carrot-fed animals
0– 100	2	2	6	13	3	3
100– 200	8	8	12	10	4	6
200– 300	6	8	10	10	5	4
300– 400	7	9	5	10	8	13
400– 500	7	12	4	8	4	9
500– 600	5	8	5	4	5	6
600– 700	4	3	8	3	6	4
700– 800	4	2	6	2	5	4
800– 900	8	6	2	2	2	7
900–1000	2	3	4	3	3	1
1000–1100	—	3	—	—	3	3
1100–1200	4	—	1	1	2	1
1200–1300	2	1	—	—	7	1
1300–1400	1	3	1	2	3	1
1400–1500	2	—	1	—	2	2
1500–1600	1	—	—	1	—	1
1600–1700	1	—	—	—	1	—
1700–1800	1	—	—	—	1	1
1800–1900	1	—	1	—	1	1
1900–2000	—	—	—	—	—	1
2000–2100	—	1	—	—	—	—
2100–2200	—	—	—	—	—	—
2200–2300	—	—	1	—	—	—
2300–2400	—	—	—	—	—	—
2400–2500	1	—	—	—	1	—
⋮	⋮					
3100–3200	—	—	—	—	1	—

As regards the secretion of carotene in the colostrum, as demonstrated by the figures recorded in the yellow units column, a very similar result to that of the secretion of vitamin A was obtained. No significant difference was observed between the distribution throughout the range of the controls and of the carrot-fed cows. A greater number of the carrot-fed cows than of the controls had a value of less than 500 yellow units per 100 ml. The two highest values recorded belonged to cows of the control group.

It is concluded, therefore, that the feeding of carrots—a carotene-rich foodstuff—at the rate of 3 lb. per day per animal from the day on which cows came off pasture until parturition did not effect the vitamin A or carotene content of the colostrum secreted.

It was considered possible that the above results might be due rather to the actual composition of the vehicle—carrots—by which the vitamin A was given to the cows than to the amount of vitamin A given. It was decided the following year to repeat the

experiment using a different medium for the administration of the vitamin, namely, cod-liver oil. Accordingly, on the same five farms and utilizing the same herds, fifty-seven cows were fed cod-liver oil and fifty cows acted as controls—the controls being again chosen so that they would calve as near as possible to the dates of those receiving cod-liver oil. The cod-liver oil was fed at the rate of $\frac{1}{7}$ pt. per day per animal. Since the potency of the cod-liver oil used was known it could be calculated that each animal received over 70,000 international units of vitamin A per day. The cod-liver oil was added to the ordinary dairy ration and fed from the day the cows came off pasture till parturition. As in the case of the previous experiment a colostrum sample was taken immediately after calving and despatched to the laboratory.

The results of the assay of these samples are recorded in Table 2.

Table 2. *Showing distribution of control and cod-liver-oil-fed cows according to the content of vitamin* A *per* 100 *ml. of their colostrum*

	Blue units (Moore's)		Yellow units (Moore's)		International units	
Units per 100 ml.	No. of control animals	No. of C.L.O.-fed animals	No. of control animals	No. of C.L.O.-fed animals	No. of control animals	No. of C.L.O.-fed animals
0– 100	—	—	5	6	—	—
100– 200	3	4	3	13	2	3
200– 300	7	7	14	10	3	8
300– 400	8	6	7	12	5	7
400– 500	5	7	7	5	8	4
500– 600	6	6	2	3	7	7
600– 700	3	3	4	2	6	6
700– 800	4	10	2	2	4	5
800– 900	4	2	1	1	1	1
900–1000	1	4	1	1	1	4
1000–1100	1	1	2	—	2	2
1100–1200	4	1	—	2	2	4
1200–1300	—	—	1	—	4	2
1300–1400	1	1	—	—	1	1
1400–1500	2	1	1	—	2	1
1500–1600	—	2	—	—	—	1
1600–1700	—	—	—	—	—	—
1700–1800	—	1	—	—	—	—
1800–1900	1	—	—	—	—	—
1900–2000	—	—	—	—	—	1
2000–2100	—	—	—	—	—	—
2100–2200	—	—	—	—	—	—
2200–2300	—	—	—	—	—	—
2300–2400	—	—	—	—	—	—
2400–2500	—	—	—	—	—	—
2500–2600	—	—	—	—	—	—
2600–2700	—	—	—	—	—	—
2700–2800	—	1	—	—	—	—

As in the case of the carrot-feeding experiment little difference is discernible between the range of the vitamin A content of the colostrum of the cod-liver-oil-fed group and of the control group with the exception of one member of the cod-liver-oil-fed group whose colostrum had the very high value of over 2700 blue units per 100 ml. The frequency distribution of the two groups is so similar that the only conclusion that can be drawn is that the feeding of cod-liver oil throughout the stall-feeding period before parturition did not increase the vitamin A content of the colostrum. Study of the yellow units column of Table 2 shows that even the carotene content of the colostrum was not influenced by the feeding of the cod-liver oil. The cows secreting the two greatest concentrations of carotene belonged to the control group, and the colostrum of the majority

of the cows in the cod-liver-oil-fed group had a carotene content of less than 500 units per 100 ml.

Thus we have failed to show that feeding vitamin A-rich concentrates to dairy cows during stall feeding, when their rations are deficient in vitamin A, has altered the vitamin A or carotene content of their colostrum. This result has been obtained despite the feeding being continued from the day on which the cows were taken off pasture in October or November till parturition took place in January, February, March or April. It was considered that the vitamin A content of the supplement was adequate to supply the total amount secreted in the milk during the early period of stall feeding and to enhance greatly the body reserves of vitamin A during the dry period before parturition. Thus at parturition the reserves of the carrot-fed and cod-liver-oil-dosed cows ought to have been extremely high as compared with those of the control groups, since these cows were on a very low vitamin diet during the whole period of the experiment, and during the period of lactation must have depleted their reserves, which could not have been replaced during the dry period because of the low vitamin content of the diet. Consequently if the vitamin A content of the colostrum depends on the liver reserves then the vitamin A content of the colostrum of the experimental cows should have been extremely high as compared with those of the controls, but this was found not to be the case. It must be concluded, therefore, that either the feeding of carrots and cod-liver oil did not keep the liver reserves of vitamin A at a high level or else that the vitamin A content of the colostrum bears no relationship to the liver reserves of the cow. It would seem that only by a most expensive experiment, in which all the cows were killed at parturition, could this point be settled. It has been shown by many research workers that the vitamin A and carotene content of milk and butterfat is increased considerably when cows are allowed access to pasture after stall feeding of dry rations or when their winter rations are supplemented by grass silage or dried grass. Therefore the most likely explanation of the results of the present experiment is that the vitamin A content of the colostrum as distinct from milk is unaffected by either the vitamin A content of the diet being fed at parturition or by the amount of the vitamin A reserves in the body.

Since we failed to increase the vitamin A content of the colostrum by increasing the vitamin A content of the diet of the cow and thus failed to ensure that every calf in a herd was receiving an adequate supply of vitamin A, there are now in progress experiments which have been designed to show whether dosing the calf at birth with a high concentrate of vitamin A would increase its resistance to white scour and allied diseases. These experiments will be reported on at a later date.

We are indebted to Dr J. Russell Greig, Director of the Institute, for his continued interest and assistance in the work and to the British Cod-Liver-Oil Producers (Hull) for the supply of the cod-liver oil used in the experiments.

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(1) Stewart, J. & McCallum, J. W. (1938). *J. agric. Sci.* **28**, 428.
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(*MS. received for publication* 31 *March* 1942)

637.143:576.8

293. BACTERIOLOGICAL ASPECTS OF THE MANUFACTURE OF SPRAY-DRIED MILK AND WHEY POWDERS, INCLUDING SOME OBSERVATIONS CONCERNING MOISTURE CONTENT AND SOLUBILITY

By E. L. CROSSLEY and W. A. JOHNSON, *Central Laboratories, Aplin and Barrett Ltd., Yeovil, Somerset*

A review of existing knowledge concerning the bacteriology of milk powders has been published by Allen(1), but most of the available literature deals only with roller-dried powder, which differs from spray-dried in physical, chemical, and bacteriological conditions owing to wide differences in the two methods of manufacture. In a preliminary paper by one of us(2) a general outline was given of the bacteriological features observed during the first two years of operation of two spray-drying plants. Shortly afterwards Nichols(3) described an extensive investigation of the flora of spray-process milk powder produced by eight factories, and showed that wide variations in bacteriological quality were found between the products of different factories and of the same factory; such variations might be seasonal, daily, or even hourly.

The main purpose of the present investigation was to elucidate the factors which control the bacterial flora of spray-dried powders, to ascertain the causes of any observed variations, to record the chief bacteriological features of the manufacturing process, and to suggest possible control methods. The work was carried out with two large plants of the Kestner type, in which the milk is precondensed by means of triple effect climbing film evaporators, and is dried by spraying into a hot-air chamber in the form of a fine mist produced by means of a rotating disk atomizer (Fig. 1). The technical considerations involved have been discussed by Philip(4) and Scott(5). Prior to the above processing, the milk is submitted to high temperature-short time pasteurization by continuous passage through a tubular heater. The two plants are identical, but one was operated with a raw milk supply of good quality and the other with a very poor supply.

Experimental methods

(1) *Sampling*

As preliminary work had shown that wide variations commonly occurred in the same plant, it was considered essential to sample the same milk at the different stages of its passage through the plant, as far as this was practicable when dealing with a continuous flow of large volumes of milk. Whilst absolute accuracy in this respect is only possible with the first milk through the plant, attempts were made to ensure representative sampling at other times by accurately timing the various processes, followed by collection of samples by several workers employing stop watches.

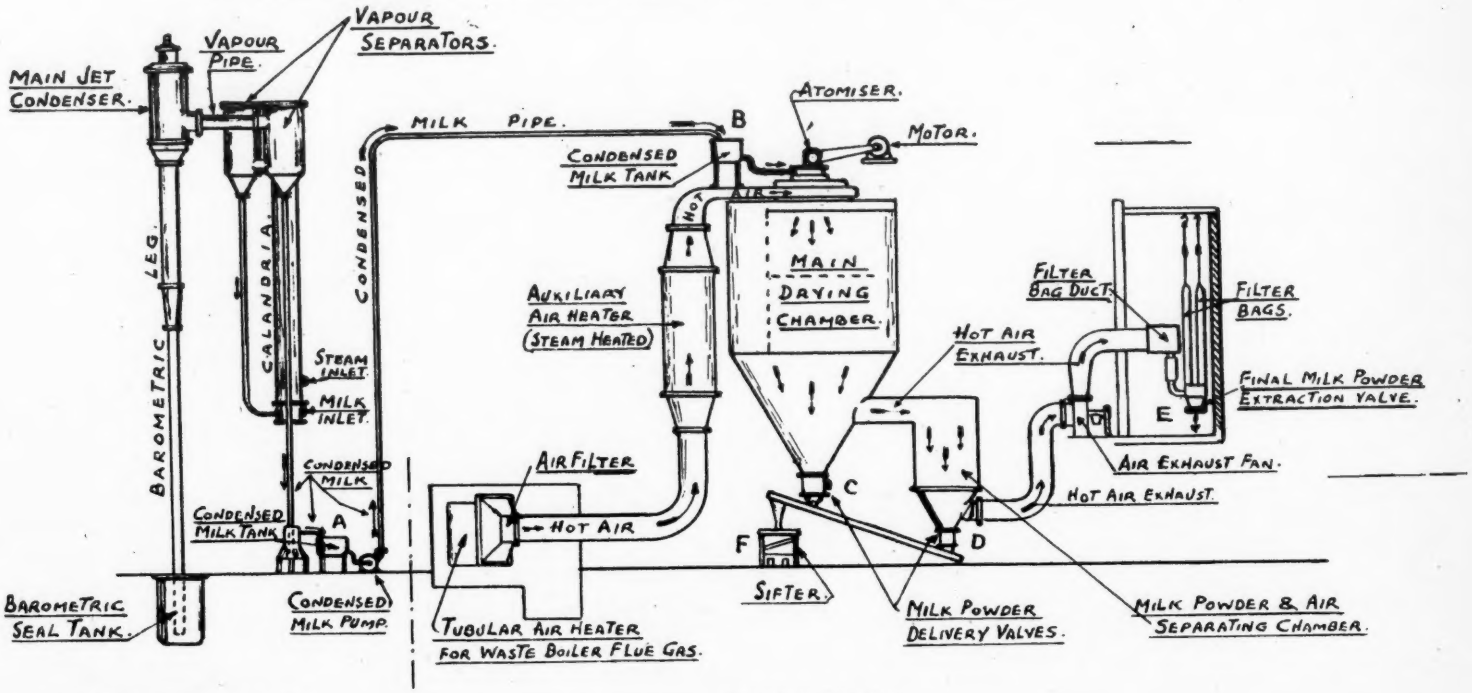
KESTNER "SPRAY PROCESS" MILK DRYING PLANT.
MAIN JET CONDENSER.
VAPOUR PIPE.
VAPOUR SEPARATORS.
BAROMETRIC LEG.
CALANDRIA.
STEAM INLET.
MILK INLET.
CONDENSED MILK.
CONDENSED MILK TANK.
A
BAROMETRIC SEAL TANK.
CONDENSED MILK PUMP.
CONDENSED MILK PIPE.
B
ATOMISER.
MOTOR.
CONDENSED MILK TANK.
HOT AIR
AUXILIARY AIR HEATER (STEAM HEATED)
AIR FILTER
HOT AIR
TUBULAR AIR HEATER FOR WASTE BOILER FLUE GAS.
MAIN DRYING CHAMBER.
C
F
SIFTER.
D
MILK POWDER DELIVERY VALVES.
FILTER BAG DUCT.
FILTER BAGS.
HOT AIR EXHAUST.
FINAL MILK POWDER EXTRACTION VALVE.
E
AIR EXHAUST FAN.
HOT AIR EXHAUST.
MILK POWDER & AIR SEPARATING CHAMBER.
CONDENSING PLANT.
DRYING PLANT.

Fig. 1.

(2) *Bacteriological examinations*

Samples of powder were reconstituted in sterile tap water and examined by the methods commonly employed for milk samples. Preliminary work soon showed, however, that very wide discrepancies were obtained by different workers, or even by the same worker, in plate counts of the same sample. The main source of error was traced to the reconstitution process, and it was demonstrated that variations in the degree of shaking could materially influence the plate count obtained; the adoption of a standard method was found essential. The following procedure yielded satisfactory results, with little greater variation than is normally obtained between plate counts of normal milk.

10 g. of powder were weighed out into a sterile glass-stoppered weighing bottle, using aseptic precautions. The powder was then readily transferred to a glass-stoppered bottle of about 200 ml. capacity, containing 90 ml. sterile tap water. Solution was obtained by vigorous shaking through a vertical motion of about 18 in., exactly 75 shakes being counted. The reconstituted milk was then allowed to stand for 5 min. in order to disperse froth before commencing the bacteriological analysis. Owing to the ready solubility of the powders under investigation, devices to aid solution, such as shaking with lead shot, or the use of lithium hydroxide, were found unnecessary. 1 ml. of the solution was assumed to be equivalent to 0·1 g. of powder and thus became the 1/10 dilution. Further dilutions down to 1/10,000 were then made in the normal manner, using 9 ml. quantities of sterile Ringer's solution.

Plate counts were determined on standard milk agar at 37° C., employing incubation for 3 days, 2 days being insufficient. 1 ml. of the reconstituted milk was plated on whey agar adjusted to *p*H 3·5 with citric acid, and incubated at 22° C. to obtain a yeast and mould count. All plate counts were recorded per 1 g. of powder. At least 20 ml. of reconstituted milk were inoculated into Maconkey broth for the presumptive coliform test; quantities less than 10 ml. almost invariably yielded negative results. Presumptive positives were further examined by plating out on eosin methylene blue agar, followed by the usual confirmatory tests(6) on isolated cultures. Spore-forming anaerobic bacilli were detected by inoculation of 5 ml. reconstituted milk into a milk-peptone-meat medium described by Crossley(7), followed by heating for 15 min. at 80° C. and incubation at 37° C. for 4 days.

(3) *Chemical examinations*

Determinations were made of the moisture content (by drying in a vacuum oven) and solubility index of powder samples. These properties are of commercial importance and must be considered in conjunction with the bacteriological quality when plant operation is under review.

The general flora of spray-dried milk and whey powders

Before passing to a detailed consideration of manufacturing processes, it may be useful to discuss the general flora of spray-dried powders. A series of summarized figures is presented in Table 1, and covers the results of examinations of 671 powder samples produced over a period of 4½ years. Results from the two plants are recorded separately, since plant 1 handled an extremely poor raw milk supply throughout the whole period. In an earlier paper(2) it was reported that the powder produced by this plant contained larger numbers of bacteria than the powder from plant 2, handling a fairly good milk

supply. This statement was based on results obtained during the early period of operation of both plants, before close bacteriological control had been established, and such findings were therefore to be expected.

Subsequently, close attention was devoted to the operation of plant 1, with the result that a complete reversal occurred. Over the whole period, plant 1 with the poorer milk supply showed a mean plate count for separated milk powder of 57,790 per g., as compared with a mean of 198,100 for plant 2. Both plants were operated mainly on separated milk, to which the bulk of the figures refer. The smaller number of figures referring to full-cream milk powder indicate that with plant 1 there was a considerably higher mean count than for separated milk powder; with plant 2 the full-cream powders show a slightly lower mean count. Observations during the final year showed that at both plants, with close control in operation, the general tendency was to obtain higher plate counts for full-cream powder.

A striking feature was the very wide variation in numbers of bacteria, extending from 200 up to 19,500,000 per g. in the case of separated milk powder produced by plant 1; the frequency distribution of these figures is shown in Table 20. The figures include the early period of operation of both plants, and results during the last two years showed a much narrower range of variation and lower mean counts. Nichols(3) has reported much wider variations and higher mean counts than were recorded in this investigation, and appreciation of the underlying causes is obviously of vital practical importance. In general, the plate counts of spray-dried whey powder (Table 1) were very low, although higher than those of roller-dried whey powder. Comparatively few samples were, however, examined, since the spray process was not regularly employed for whey drying until the spring of 1941. For comparative purposes it may be recorded that eighteen samples of roller-process whey powder, manufactured during the same period, showed a mean plate count of 330 per g.

It has previously been reported(2) that acid-forming thermoduric cocci form the bulk of the flora of spray-dried milk powder, and subsequent observations have confirmed this view. Indeed, it seems evident that the flora is of a highly specialized type and confined to comparatively few species. Detailed studies of the organisms which developed on agar plates at 37° C. were not made for every sample examined, though the general picture presented appeared very constant. At intervals, however, plates were selected at random and each visible type of colony picked off for detailed examination. The findings may be summarized as follows:

Cocci

(*a*) *Streptococci.* Most of the colonies examined proved to be streptococci, which were classified according to the scheme of Sherman(8). All species fell into either the '*viridans*' or '*enterococcus*' divisions; no '*pyogenic*' or '*lactic*' species were isolated.

The species confirmed were *Str. durans* (40 strains), *Str. thermophilus* (13 strains), *Str. bovis* (5 strains), *Str. faecalis* (3 strains), and *Str. liquefaciens* (2 strains), all of which survived laboratory pasteurization at 145° F. for 30 min.

It was observed that very high powder counts were commonly associated with *Str. thermophilus* strains, whilst Nichols(3) recorded that *Str. thermophilus* occurred more frequently than *Str. durans*. The powder counts met with by the authors were generally much lower than those reported by Nichols. Recently the authors have examined spray-

Table 1. *Summarized bacteriological figures for all powder samples*

Type of powder	Plant	No. of samples	Plate count at 37° C.			Coliform positive samples		Spore-forming anaerobes		Yeast- and mould-positive samples		
			Mean from mean log	Range of counts								
				Min.	Max.	No.	%	No.	%	No.	%	Mean count from mean log
Separated milk	1	411	57,790	200	19,500,000	96	23·2	57	13·8	55	13·4	25
Full-cream milk	1	31	444,700	2,000	9,680,000	18	58·1	4	12·9	8	25·8	19
Separated milk	2	150	198,100	11,600	5,200,000	33	22·0	8*	15·7	11*	21·6	60
Full-cream milk	2	49	136,000	2,100	3,230,000	10	24·8	4†	21·1	3†	15·8	90
Whey	2	30	29,680	3,500	600,000	10	33·3	4	13·3	4	13·3	302
All types		671				167	24·9	77	14·2	81	14·9	

* 51 samples tested. † 19 samples tested.

Table 3. *Summarized figures of plant samples. Plant* 1

Stage of manufacture	No. of samples	Mean log plate count at 37° C.	Corresponding actual count	Coliform organisms, no. of samples							Spore-forming anaerobes, no. of positive samples	Yeast- and mould-positive samples	
				Absent 1 ml.	+ 1 ml.	+ 0·1 ml.	+ 0·01 ml.	+ 0·001 ml.	+ 0·0001 ml.	+ 0·00001 ml.		No.	Mean count (from logs)
Raw milk	18	7·5748	37,570,000	Nil	Nil	Nil	2	3	1	12	No test	No test	—
Raw milk, laboratory pasteurized	18	4·0827	12,100	16	1	1	Nil	Nil	Nil	Nil	No test	No test	—
Separated milk	23	7·3270	21,230,000	Nil	Nil	Nil	2	2	3	16	No test	No test	—
Separated milk, laboratory pasteurized	21	4·8504	70,860	20	1	Nil	Nil	Nil	Nil	Nil	No test	No test	—
Pasteurized separated milk	34	4·8684	73,860	26	2	3	2	Nil	1	Nil	No test	—	—
Evaporated separated milk, leaving evaporator	33	5·7148	518,570	8	1	9	5	7	Nil	3	3	8	7
Evaporated separated milk, entering atomizer	24	6·4470	2,799,000	1	1	3	7	11	Nil	1	4	14	11
Separated milk powder	32	5·0390	109,400	32*	Nil	Nil	Nil	Nil	Nil	Nil	2	3	20 (per g. of powder)

* 5 samples positive in 10 ml. reconstituted milk; 1 sample positive in 20 ml. reconstituted milk; 26 samples negative in 20 ml. reconstituted milk.

Table 2. *Corresponding plant samples*

Run	Plate count 37° C.	Coliform organisms ml.	Plate count 37° C.	Coliform organisms ml.	Plate count 37° C.	Coliform organisms ml.	Spore-forming anaerobes	Yeasts and moulds per ml.
			Plant 1. Separated milk					
	Raw skim milk		Pasteurized skim milk		Evaporated skim milk leaving evaporator			
1	—	—	1,532,000	0·01	3,200,000	0·1	—	—
2	—	—	80,000	1	2,900,000	0·1	—	—
3	—	—	1,751,000	0·1	2,568,000	0·001	—	—
4	—	—	1,467,000	0·1	1,212,000	0·001	—	300
5	25,200,000	0·00001	3,100	–	19,500	–	—	—
6	127,800,000	0·00001	642,000	0·1	750,000	0·1	—	—
7	—	—	2,200	–	—	–	—	—
8	1,120,000	0·00001	37,000	–	300,000	0·001	—	—
9	—	—	402,800	–	6,770,000	0·1	—	—
10	—	—	910,000	1	16,400,000	0·00001	—	—
11	—	—	223,300	–	35,000	0·01	*Welchii* +	—
12	—	—	19,000	–	3,132,000	0·001	—	—
13	—	—	641,100	–	3,190,000	–	—	—
14	2,600,000	0·01	8,700	–	38,000	0·01	—	—
15	80,800,000	0·00001	141,700	–	—	–	—	—
16	—	—	201,400	–	1,710,000	–	—	—
17	—	—	—	–	—	–	—	—
18	2,000,000	0·001	176,000	0·01	—	–	—	—
19	—	—	4,200	–	462,000	0·1	*Putrificum* +	3
20	181,100,000	0·00001	360,000	–	145,000	0·1	—	—
21	15,600,000	0·00001	4,000,000	0·00001	4,154,000	0·001	—	2
22	—	—	12,800	–	81,600,000	0·00001	—	—
23	—	—	5,000	–	339,000	–	—	—
24	49,920,000	0·00001	4,900	–	35,000	–	—	—
25	—	—	—	–	132,000	–	*Welchii* +	—
26	—	—	670	–	550,000	0·01	—	102
27	50,000,000	0·00001	758,000	–	156,000	0·00001	—	—
28	—	—	480	–	147,000	0·01	—	—
29	—	—	128,400	–	91,000	0·1	—	4
30	33,700,000	0·00001	66,000	–	30,000	–	—	—
31	—	—	186,500	–	234,000	0·01	—	—
32	—	—	129,000	–	141,000	0·001	—	—
33	—	—	30,000	–	30,422,000	0·001	—	—
34	14,500,000	0·00001	216,000	–	—	–	—	—
35	47,000,000	0·00001	84,000	–	—	–	—	—
36	4,760,000	0·00001	14,800	–	—	–	—	—
37	—	—	—	–	796,000	1	—	6
38	—	—	—	–	212,000	0·1	—	1
39	—	—	—	–	117,000	–	—	—
40	—	—	—	–	50,000	0·1	—	2
			Plant 1. Full-cream milk					
	Raw whole milk		Pasteurized whole milk		Evaporated milk leaving evaporator			
41	84,000,000	0·00001	6,900	1	116,000	0·1	—	—
42	52,000,000	0·00001	176,000	0·1	1,630,000	0·001	—	—
			Plant 2. Separated milk					
	Raw skim milk		Pasteurized skim milk		Evaporated skim milk leaving evaporator			
43	1,320,000	0·001	61,000	0·1	22,500,000	0·0001	—	40
44	—	—	12,100	–	64,800	1	*Welchii* +	—
45	5,680,000	0·0001	10,500	–	135,000	0·01	*Putrificum* +	—
46	12,000,000	0·0001	280	1	1,300,000	0·01	—	4
47	1,840,000	0·001	180,000	0·01	11,325,000	0·001	—	115
			Plant 2. Full-cream milk					
	Raw whole milk		Pasteurized whole milk		Evaporated milk leaving evaporator			
48	1,200,000	0·01	5,200	–	16,900	0·001	—	—
			Plant 2. Whey					
	Raw whey		Pasteurized whey		Evaporated whey leaving evaporator			
49	137,000,000	0·00001	14,000	0·1	354,000	0·001	—	1
50	17,600,000	0·00001	76,000	–	700,000	–	—	—
51	32,000,000	0·00001	240,000	–	108,000	–	—	1
52	7,500,000	0·0001	10,100	–	75,000	0·01	—	85
53	13,000,000	0·00001	1,620	–	406,000	0·0001	—	79

*taken at each stage of manufacture.**

Plate count 37° C.	Coliform organisms ml.	Spore-forming anaerobes	Yeasts and moulds per ml.	Plate count 37° C.	Coliform organisms g.	Spore-forming anaerobes	Yeasts and moulds per g.	Run
Plant 1. Separated milk								
Evaporated milk entering atomizer				Skim-milk powder				
—	–	—	—	3,600,000	1	—	—	1
4,813,000	0·001	—	—	1,810,000	1	*Welchii*	—	2
—	–	—	—	1,452,000	–	—	—	3
1,218,000	0·001	—	250	1,222,000	–	—	—	4
6,360,000	0·01	—	9	1,208,000	1	—	—	5
4,000,000	0·01	—	1	1,180,000	–	—	40	6
—	–	—	—	660,000	–	—	—	7
—	–	—	—	640,000	–	*Putrificum* +	—	8
35,700,000	0·01	—	3	604,600	–	—	—	9
36,600,000	0·00001	—	11	469,000	–	—	—	10
12,560,000	0·001	*Putrificum* +	—	362,000	–	—	—	11
—	–	—	—	312,000	–	—	—	12
39,700,000	0·001	—	4	200,000	–	—	—	13
—	–	—	—	184,000	1	—	—	14
—	–	—	—	160,000	–	—	—	15
3,914,000	0·1	*Butyricum* +	—	143,000	2	—	—	16
2,320,000	0·01	—	—	127,000	–	—	20	17
—	–	—	—	101,300	–	—	—	18
—	–	—	—	90,000	–	—	—	19
970,000	0·001	—	50	58,100	–	—	—	20
137,600,000	0·001	—	30	44,000	–	—	—	21
—	–	—	—	36,000	–	—	—	22
19,900,000	0·01	—	15	31,200	–	—	—	23
—	–	—	—	25,100	–	—	—	24
342,000	1	*Welchii* +	—	22,100	–	—	—	25
14,000,000	0·01	—	4	16,500	–	—	—	26
—	–	—	—	16,000	1	—	—	27
1,700,000	0·001	—	—	11,900	–	—	—	28
244,000	0·001	—	37	8,000	–	—	—	29
33,800	0·01	—	—	5,500	–	—	—	30
870,000	0·1	*Putrificum* +	—	3,400	–	—	—	31
11,800,000	0·001	—	6	1,100	–	—	10	32
—	–	—	—	—	–	—	—	33
—	–	—	—	—	–	—	—	34
—	–	—	—	—	–	—	—	35
—	–	—	—	—	–	—	—	36
1,056,000	0·1	—	3	—	–	—	—	37
1,030,000	0·001	—	11	—	–	—	—	38
250,000	–	—	—	—	–	—	—	39
122,000	0·001	—	—	—	–	—	—	40
Plant 1. Full-cream milk								
Evaporated milk entering atomizer				Full-cream powder				
107,000	0·001	—	1	726,000	–	—	—	41
3,000,000	0·0001	—	1	440,000	1	—	—	42
Plant 2. Separated milk								
Evaporated milk entering atomizer				Skim-milk powder				
39,400,000	0·0001	—	280	1,930,000	2	—	—	43
95,100	0·1	*Welchii* +	—	137,300	1	—	—	44
720,000	0·0001	—	—	102,000	1	—	—	45
1,100,000	0·001	—	2	79,000	1	—	—	46
—	–	—	—	—	–	—	—	47
Plant 2. Full-cream milk								
Evaporated milk entering atomizer				Full-cream powder				
151,000	0·0001	*Butyricum* + and *Welchii* +	—	186,000	1	*Butyricum*	—	48
Plant 2. Whey								
Evaporated whey entering atomizer				Whey powder				
420,000	0·001	*Butyricum* +	90	400,000	1	—	70	49
1,600,000	–	—	1	210,000	–	—	—	50
110,000	–	No test	1	37,100	–	—	—	51
—	0·01	—	122	32,700	–	—	—	52
183,000	0·001	—	49	3,500	1	—	—	53

* Results arranged in descending order of powder plate counts.

dried separated milk powders imported from the U.S.A., and in these the '*viridans*' streptococci constituted a high proportion of the flora, with *Str. bovis* the predominant species.

(*b*) *Micrococci.* Micrococci were also found regularly in spray-dried powders. The species isolated most frequently were *M. freudenreichii*, *M. varians*, *M. candidus*, *M. mucofaciens*, and *M. luteus*. Although thermoduric strains of all these species are known, the cultures isolated from powders all failed to survive laboratory pasteurization at 145° F. for 30 min. Gibson & Abdel-Malek(9) found that *M. luteus* was the commonest micrococcus in milk pasteurized at 63° C. but the authors have not observed this in the case of spray-dried milk powder.

Hileman, Leber & Speck(10) found that certain micrococci survive high temperature-short time pasteurization more readily than low temperature-holder pasteurization, and list *M. candidus*, *M. epidermidis*, *M. luteus*, and *M. varians* as the common species in milk pasteurized by the high-temperature method. It is uncertain, therefore, whether the micrococci encountered in powders were survivals of the pasteurization process or whether they gained access at some post-pasteurization stage (e.g. evaporation) and survived spray drying.

Non spore-forming bacilli

(*a*) *Achromobacter types.* Three *Achromobacter* species were isolated fairly frequently from the milk-agar plates, and were classified as *Achr. acidum*, *Achr. centropunctatum*, and *Achr. geniculatum*. All the cultures were killed by laboratory pasteurization at 145° F. for 30 min. These organisms no doubt arose from general plant contamination, probably between pasteurization and drying.

(*b*) *Coliform organisms.* Early in this investigation it was observed that 'false positives' in Maconkey broth were of common occurrence, and the complete figures showed that such false positives, yielded by anaerobes, constituted 55% of the total presumptive positives in plant 1, and 29% in plant 2. When samples of stored powder were examined 79% false positives were found. It was concluded, therefore, that use of the 'presumptive coliform' test, without further confirmation, might yield misleading results. The summarized figures in Table 1 which include only confirmed positives, show that coliform organisms were present in 24·9% of all samples; the separate classes of powder varied little from this figure, with the exception of full-cream milk powder produced by plant 1, which yielded 58·1% of coliform-positive samples. It was realized that the examination of 20 ml. of reconstituted milk constituted a stringent test, and although a number of positives were obtained from 10 ml. the examination of 1 ml. only (as in testing milk samples) yielded almost wholly negative results. Nevertheless, the results showed that very small numbers of coliform bacteria may survive in a fair proportion of spray-process powders, and for certain purposes, e.g. ice-cream manufacture, their presence is not desirable. The effect of manufacturing processes on the incidence of coliform organisms will be discussed later.

Confirmatory tests were carried out on 159 true positives, from which 198 cultures were isolated. Only one strain was found which could not be exactly placed in the usual classification of coliform types(6); this strain, represented by sixteen cultures, appeared to be *aerogenes type I* in all respects except for a negative Voges-Proskauer reaction. This reaction (using O'Meara's method) remained negative in spite of repeat tests using varying

periods of incubation of the glucose-phosphate broth. The results of all tests may be summarized as follows:

Result of confirmatory tests	Number of presumptive positives	
False positives due to anaerobes	161	50·3% of total presumptive positives
Coli type I	48	
Coli type II	7	
Intermediate type I	2	
Intermediate type II	2	
Aerogenes type I	29	
Aerogenes (V.P. negative) type I	3	Total 120, 75·5% of true positives
Aerogenes type II	1	
Irregular type III	1	
Irregular type V	3	
Irregular type VIII	2	
Cloacae	22	
Mixture of *coli* I and *aerogenes* I	13	
" *coli* I and *aerogenes* I (V.P. negative)	2	
" *coli* I and *aerogenes* II	1	
" *coli* I and Intermediate I	1	Total 39, 24·5% of true positives
" *coli* I and *cloacae*	5	
" *aerogenes* I and *cloacae*	5	
" *aerogenes* I (V.P. negative) and *cloacae*	11	
" intermediate I and *cloacae*	1	
	320	

The 159 true positives were classified as follows, and were compared with the *coli* confirmation results obtained from 133 samples of raw milk, all of which were true positives.

Type	Powders		Raw milk	
	No. of cultures	% of cultures	No. of cultures	% of cultures
Coli I	70	35·4	90	54·8
Coli II	7	3·5	5	3·0
Intermediate I	4	2·0	6	3·6
Intermediate II	2	1·0	3	1·8
Aerogenes I	47	23·7	44	26·8
Aerogenes I (V.P. negative)	16	8·1	Nil	—
Aerogenes II	2	1·0	3	1·8
Irregular III	1	0·5	Nil	—
Irregular V	3	1·5	5	3·0
Irregular VIII	2	1·0	4	2·4
Cloacae	44	22·2	4	2·4
	198		164	

Thus of the individual coliform types isolated from powders, *coli* I occurred most frequently, with *aerogenes* types almost equally common. The incidence of *coli* I was, however, appreciably less than in raw milk, and it seems doubtful whether the presumptive coliform test has any direct bacteriological significance when applied to milk powder, owing to the occurrence of false positives and the preponderance of non-intestinal strains among the true positives.

Perhaps the most noticeable feature was the isolation of 22·2% *cloacae* strains, as compared with 2·4% from raw milk: Wilson(6) recorded 3·7% *cloacae* strains from raw milk. The *aerogenes*, intermediate, and irregular strains also occurred with slightly greater frequency in powders than in raw milk.

Comparisons of confirmatory tests obtained from powders produced by the two plants did not differ sufficiently to justify separate tabulation. It has been noted that powders produced by plant 2 from a good milk supply showed a smaller proportion of false positives; in addition, the true positives from these powders showed a smaller proportion

of samples containing more than one coliform strain, whilst *coli* I strains were rather less frequent and *aerogenes* I strains more frequent than in plant 1 powders.

Laboratory pasteurization of the strains in separated milk at 145° F. for 30 min. showed that 25·0% *coli* I, 38·4% *aerogenes* I, and 16·6% *cloacae* strains from powders were heat resistant; 48·5% of all strains were heat resistant. None of the other coliform types was found to be heat resistant, but the total number of such cultures obtained was not large. Only two heat-resistant strains, both *coli* I, were found in the raw-milk cultures, i.e. 2·2% of *coli* I strains. Heat-resistant *coli* I strains are now well known, but heat-resistant strains of other coliform types are met with less commonly. These results, together with the relative frequencies of types already discussed, suggest that the coliform flora of powders is not derived directly from the raw-milk supply, but is partly a consequence of plant contamination by certain heat-resistant strains. The special conditions of the spray-drying process do in fact provide opportunities for such contamination, which will be discussed later.

Spore-forming bacilli

(*a*) *Aerobic species.* As would be expected, aerobic spore-forming bacilli of the *B. subtilis* group were of regular occurrence, but their numbers rarely exceeded a few hundreds per g. of powder. No detailed classification of these species was made.

(*b*) *Anaerobic species.* As will be seen from Table 1, spore-forming *anaerobes* were found in 14% of all powder samples, and the five separate classes of powder varied very little from this mean figure. In view of the sensitive method of testing employed, it seemed evident that the number of such organisms was very small, and that they would be unlikely to cause spoilage of the reconstituted milk. Nevertheless, some users of milk powder object to their presence on the same general grounds as the presence of coliform organisms. A rough classification of general types, employing the method described by Crossley(7), showed that acid-fermenting types predominated over the putrefactive species, as follows:

	Welchii type	*Butyricum* type	*Sporogenes* or *putrificum* type	Total
Plant 1	24	16	21	61
Plant 2	6	5	5	16
	30	21	26	77

Since all these species form extremely heat-resistant spores, control methods must be based upon improvement of the raw-milk supply.

Yeasts and moulds

Yeasts or moulds were found in 14·9% of all powder samples (Table 1), but except in whey powder the actual numbers were negligible. The percentage of positives varied little between the two plants or with the type of powder manufactured, but positive samples from plant 2 showed a slightly higher mean count.

No yeasts were found in any of the fifty samples of full-cream milk powder, and they were detected in only five of 411 samples of separated milk powder from plant 1, and in two out of fifty-one similar samples from plant 2. The figures given for milk powders therefore refer mainly to mould content, chiefly *Penicillium* species and (less commonly) *Oospora* or *Mucor*. In practice the general run of mould counts was lower than the mean counts would suggest.

Whey powder apparently differed from the milk powders, although only thirty samples were examined. All of these were mould negative, but four of the thirty samples contained yeasts and showed a relatively high mean count of 302 per g. Since all the samples were taken direct from the main dryer discharge, the mould and yeast counts represented actual survivals of the drying process. Unless precautions are taken, further atmospheric contamination may occur during packing processes.

Summary of powder flora

The flora of freshly made spray-dried powders therefore consisted essentially of certain streptococci and micrococci, representing comparatively few species. Aerobic spore-forming bacilli and a few *Achromobacter* species occurred regularly but only as a rule in small numbers which were unlikely to be of much practical significance. Coliform organisms, anaerobic spore-forming bacilli, and a few mould species occurred sporadically in extremely small numbers, whilst yeasts were rare; none of these organisms appeared to be a likely cause of spoilage of the reconstituted milk in comparison with the very much greater population of acid-forming cocci.

The powder flora appeared to be similar at both plants, whether separated milk, full-cream milk, or whey was dried.

All the streptococci and some of the coliform species were thermoduric, but the *Achromobacter* species, some of the coliform species, and probably the micrococci, were non-thermoduric.

MANUFACTURING PROCESSES

Individual manufacturing processes may now be considered in detail. Bacteriological data obtained during a number of plant runs are shown in Table 2; the samples represented as nearly as possible the same bulk of milk or whey. This work was carried out mainly on separated milk at plant 1, since this plant was operating under the greatest difficulties in handling a very large volume of milk of low hygienic standard. The data obtained from corresponding samples of separated milk at plant 1 are summarized in Table 3, and only these data have been employed for calculation of statistical values (Table 5) and the dot diagram in Fig. 2.

Summarized data for plant 2 (shown in Table 4) differ in that a number of random samples have been included and the figures are therefore of a general character. (Plant 2 trials wherein special care was taken to obtain corresponding samples of milk or whey are recorded in Table 2.)

Other series of samples designed to yield data concerning the effects of pasteurization and drying temperatures, hourly and seasonal variations, packing, and storage were also confined to the handling of separated milk at plant 1. This course was followed in order to eliminate possible variations due to different methods of handling or differences in milk supplies between the two plants. Moreover, the results obtained were applicable to the most difficult conditions of operation.

Samples were collected at various periods throughout the daily run and at different seasons of the year, although sampling was reduced during the winter months owing to restricted operation of the plants.

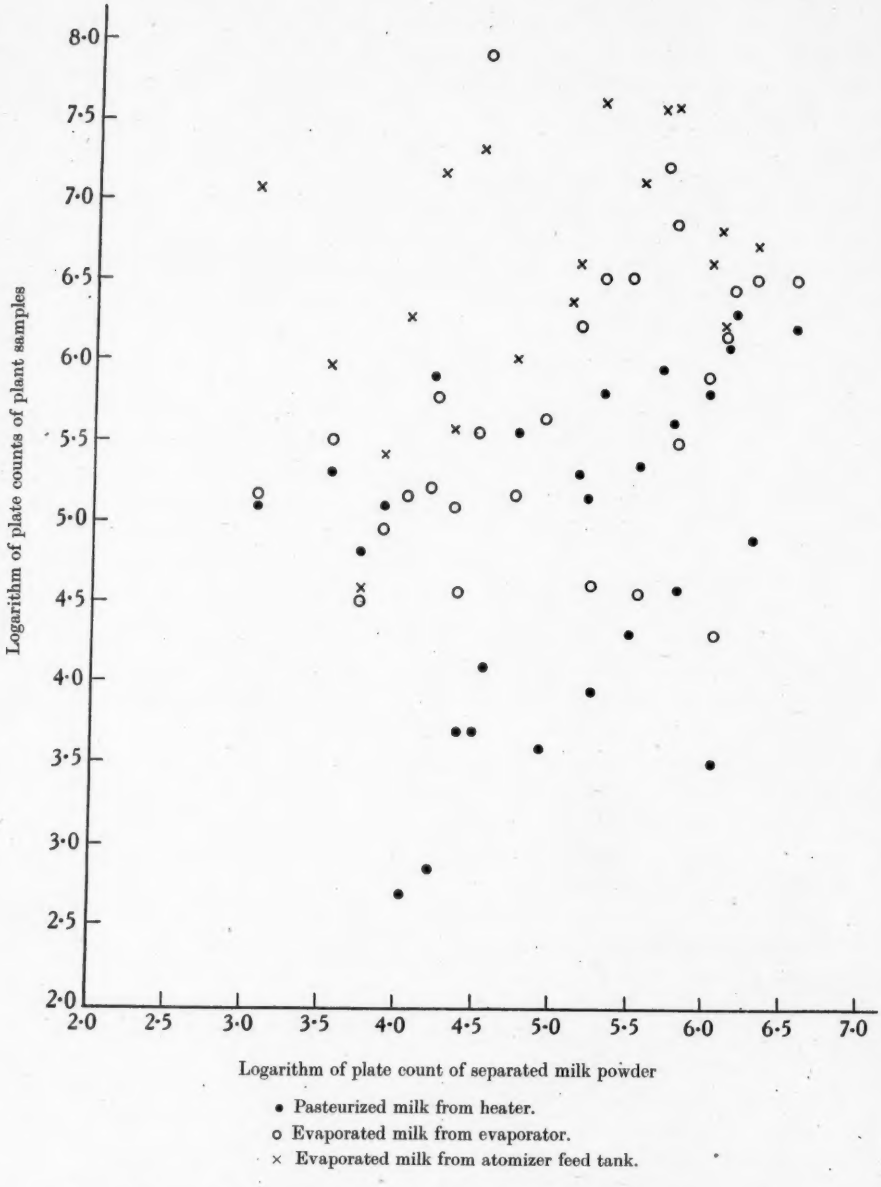

Fig. 2. Plate counts of corresponding samples of separated milk from pasteurizer, evaporator, and atomizer feed, plotted against plate counts of the corresponding samples of spray-dried powder.

(1) *Handling of raw milk*

The bacterial content of bulk raw milk as received averaged over 37,000,000 per ml. at plant 1 (Table 3) as against 825,000 per ml. at plant 2 (Table 4), but this wide disparity was not reflected in the plate counts of powders produced by the two plants. Nevertheless, although there appeared to be no close relationship between plate counts of raw milk and the powder produced, the bacteriological quality of the raw-milk supply was in fact of great importance. The bacteriological quality of powders was found to depend ultimately

Table 4. *Plant* 2. *Summarized figures of plant samples*

Sample	No. tested	Mean plate count 37° C. (from mean log)	Coliform-positive samples	
			No.	%
Bulk raw milk	144	825,000	144	100·0
Pasteurized milk. Full cream	13	7,100	1	7·7
Evaporated milk, leaving evaporator. Full cream	4	198,000	4	100·0
Evaporated milk, entering atomizer. Full cream	3	384,000	3	100·0
Powder. Full cream	49	136,300	10	24·8
Pasteurized milk. Separated milk	201	22,720	16	7·9
Evaporated milk, leaving evaporator. Separated milk	62	1,237,000	54	87·9
Evaporated milk, entering atomizer. Separated milk	16	1,770,000	16	100·0
Powder. Separated milk	150	198,100	33	22·0

Table 5. *Statistical values for logarithms of plate counts of plant samples*

Corresponding pairs of samples from plant 1		No. of pairs	Coefficients of variation %		Correlation coefficient	
x	*y*	*n*	*Cx*	*Cy*	*r*	*P*
Raw whole milk	Laboratory-pasteurized whole milk	18	9·5	17·2	0·72	Less than 0·01
Raw separated milk	Laboratory-pasteurized separated milk	21	8·1	15·5	0·55	Ditto
Raw separated milk	Pasteurized separated milk from plant	13	8·5	16·7	0·75	Ditto
Laboratory-pasteurized separated milk	Pasteurized separated milk from plant	11	11·0	17·0	0·74	Ditto
Laboratory-pasteurized separated milk	Separated milk powder	8	10·3	13·2	0·88	Ditto
Pasteurized separated milk from plant	Concentrated milk from evaporator	28	21·6	14·6	0·67	Ditto
Pasteurized separated milk from plant	Separated milk powder	28	22·1	18·4	0·72	Ditto
Concentrated milk from evaporator	Concentrated milk from atomizer feed tank	20	13·1	12·0	0·81	Ditto
Concentrated milk from evaporator	Separated milk powder	27	17·3	17·9	0·68	Ditto
Concentrated milk from atomizer feed tank	Separated milk powder	19	12·6	19·9	0·77	Ditto

upon the numbers and species of organisms which survived the pasteurization process, and in this the degree of success attained was influenced both by the raw-milk supply itself and by the plant methods of handling it.

Anderson & Meanwell(11) have shown that although there is no constant relationship between the bacterial counts of raw and pasteurized milk, clean methods of production on the farm greatly influence the suitability of milk for pasteurization; moreover, these workers demonstrated that rapid multiplication of heat-resistant organisms may take place if milk remains uncooled for more than 2 hr.

Similar considerations apply to the spray-drying process. The high bacterial count of bulked milk handled at plant 1 was largely due to absence of cooling at the farms, and it is significant that the correlation between plate counts of laboratory-pasteurized

separated milk and the corresponding separated milk powder (Table 5) was close ($r = 0{\cdot}88$). It was concluded that the number of heat-resistant organisms in the milk supply was a factor of major importance, whilst the total number of organisms was of less direct importance.

The spray-drying process itself may introduce further difficulties, since reception of the milk normally proceeds more rapidly than the plant can handle it. Storage of large volumes of milk is therefore inevitable, and during peak periods, when the drying plant is operated almost 24 hr. per day, the storage period may extend to several hours. It was shown that a pronounced increase in the number of heat-resistant organisms may occur during the storage period, as some of the following examples illustrate. (Samples taken from bulk storage tanks held at temperatures exceeding 50° F. Storage at 50° F. yielded satisfactory results.)

Storage of bulked raw milk before pasteurization

Storage period hr.	Temp. of milk ° F.	Initial plate count, 37° C.	Plate count after storage	Initial laboratory pasteurized count	Laboratory pasteurized count after storage
4	60	50,000,000	258,000,000	28,500	539,000
4	54	54,000,000	84,000,000	9,200	2,000,000
3	56	51,000,000	64,000,000	20,500	32,000
2	52	1,670,000	1,770,000	2,590	2,780
2	56	18,000,000	22,000,000	680	12,800
2	58	9,500,000	58,000,000	1,060	3,200
1	53	40,000,000	47,000,000	113,000	680,000

Absence of efficient arrangements for cool storage of the bulked milk may therefore result in serious failures. At these plants a number of storage tanks were employed in order to facilitate withdrawal of milk supplies in rotation, thus avoiding the danger of constantly mixing newly received milk with stored milk.

When separated milk is handled, further contamination may occur from feed tanks, pumps, pipe lines, preheaters, and the separator itself. The magnitude of this contamination appeared to be variable and was related to the general management of the factory, but increased numbers of heat-resistant organisms were frequently observed in separated milk. Thus eighteen samples of raw whole milk at plant 1 showed a mean plate count of 37,600,000 per ml., whilst the mean count of twenty-three samples of raw separated milk was 21,200,000 per ml.; the corresponding laboratory-pasteurized samples yielded mean counts of 12,100 per ml. for whole milk and 70,860 for separated milk (Table 3).

Finally, it must be recorded that although a high bacterial content in the raw-milk supply may not pass to the powder, chemical features such as acidity cannot be controlled so successfully. Powders produced by plant 1, in spite of lower bacterial counts, usually revealed higher acidities than those from plant 2; the acidity determination was in general a good indication of the nature of the raw-milk supply.

(2) *Pasteurization of milk*

Continuous high temperature-short time pasteurization was employed, with a normal temperature range between 159 and 164° F., and a time of passage through the tubular heater of 20 sec. Under the conditions of operation, this method is preferable to holder pasteurization from an engineering standpoint, and may offer bacteriological advantages, since thermophilic bacteria might readily develop if the holder process were used.

The pasteurized milk was passed to a balance tank feeding the evaporator, which resulted in a holding period of 3–5 min.

It has been shown that the efficiency of pasteurization is influenced by the heat-resistant flora of the raw milk. In addition, the effect of pasteurization temperatures was closely studied since such information was obviously of great practical importance. A number of runs were therefore made employing a range of pasteurizing temperatures, without varying the time factor.

Examinations were first made of a series of forty-eight samples of separated milk taken direct from the heater discharge at plant 1, and pasteurized at temperatures from 155 to 166° F. Tables 6 and 7 show that temperatures below 158° F. resulted in highly inefficient pasteurization, both as regards numbers of organisms and survival of coliform species. Although eleven of eighteen bacterial counts over 500,000 (log 5·70) occurred at 160° F. or lower, and four of five counts below 3200 (log 3·5) were found at 162° F. or over, never-

Table 6. *Pasteurized milk results at different temperatures*

Pasteurization temp. of milk, ° F.	No. of samples	Mean log total count at 37° C.	Corresponding actual count	Range of counts		Coliform bacteria, no. positive samples
				Min.	Max.	
Under 158	6	6·13398	1,361,400	630,000	4,000,000	6
158 and 159	6	4·56874	37,046	5,000	129,000	1
160	11	5·14380	139,260	2,200	1,856,000	3
161	7	4·64409	44,065	4,200	360,000	1
162	4	4·55681	36,042	480	678,000	Nil
163	7	4·89105	77,813	1,650	1,466,000	2
164–166	7	4·85361	71,386	2,200	807,000	3
All samples	48	4·99472	98,792	480	4,000,000	16

theless, no pronounced differences in bacterial results were apparent between 158 and 166° F.

A series of 269 samples of separated milk powder was then examined, wherein the pasteurization temperatures of the milks were accurately known. The summarized data are shown in Table 8 and the frequency distributions in Table 9; in general, they show a close similarity to the findings for pasteurized milk. Bacterial counts appeared to be distributed fairly evenly above the critical pasteurization temperature of 158° F. Below this temperature both the mean and maximum powder count showed striking increases; in addition, 53·3% of samples were coliform positive as compared with the mean of 20·8% for all samples.

Of sixteen powder counts exceeding 1,000,000, fourteen occurred when the milk was pasteurized below 162° F., and nine at temperatures below 160° F. Apart from this feature there appeared to be no significant variations with change of temperature between 159 and 163° F., which seemed to be the stationary range. Pasteurization between 164 and 167° F. probably tended to reduce the powder counts; the mean, range of variation, and frequency distribution of such counts all suggested this conclusion, although the indications were less marked than at low pasteurization temperatures. Apart from the increase below 158° F., the incidence of coliform-positive powder samples showed no relationship to milk pasteurization temperatures, whilst mould-positive samples seemed to show no correlation with pasteurization temperature.

The similarity of results shown by both the pasteurized milk and powder series below the marginal pasteurization temperature of 158° F. suggested that the bacteriological quality of powder was closely dependent upon the pasteurization process; further confirmation of this was afforded by the correlation coefficient of 0·72 for twenty-eight

Table 7. *Frequency table*

Pasteurization temp. of milk ° F.	Log of pasteurized milk count at 37° C.																
	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	Total
Under 158	—	—	—	—	—	—	—	—	—	—	—	—	—	2	3	—	1	6
158 and 159	—	—	—	—	1	—	—	1	2	—	2	—	—	—	—	—	—	6
160	—	—	—	1	—	1	—	1	—	1	2	—	1	1	1	2	—	11
161	—	—	—	—	2	—	—	—	1	2	1	—	1	—	—	—	—	7
162	1	—	—	—	—	1	—	—	—	—	—	—	—	2	—	—	—	4
163	—	—	1	1	—	—	—	—	—	1	1	1	—	1	1	—	—	7
164–166	—	—	—	1	—	—	1	—	—	2	1	—	—	2	—	—	—	7
Total	1	—	1	3	3	2	1	2	3	6	7	1	2	8	5	2	1	48

Table 8. *Influence of pasteurization temperature of milk on quality of separated milk powder*

Pasteurization temp. of milk ° F.	No. of samples	Mean log plate count at 37° C.	Corresponding actual plate count	Range of counts		Coliform-positive samples		Mould-positive samples			Mean moisture %	Mean solubility index
				Min.	Max.	No.	%	No.	%	Mean count		
155–158	15	5·59576	394,240	3,200	30,465,000	8	53·3	2	13·0	8	4·1	0·19
158 and 159	24	4·71191	51,510	200	12,156,000	6	25·0	Nil	Nil	Nil	3·9	0·16
160	69	4·68916	48,880	2,700	3,760,000	19	27·6	5	7·0	20	4·0	0·36
161	57	5·00059	100,140	1,000	2,470,000	5	8·8	6*	10·0	25	3·9	0·26
162	54	5·41700	261,220	400	358,000	6	11·1	7	13·0	14	4·0	0·31
163	24	4·69492	49,530	2,700	1,810,000	8	33·3	5	21·0	44	4·1	0·33
164–167	26	4·24896	17,740	200	375,000	4	15·4	2	8·0	15	3·8	0·42
Total	269	4·71107	51,420	200	30,465,000	56	20·8	27	10·0	23	3·97	0·32

* One sample gave a yeast count of 200 per g.

pairs of corresponding pasteurized milk and powder samples (Table 5). The general relationship is also indicated by the diagram in Fig. 2.

The poor milk supply received at plant 1 yielded pasteurized separated milk of rather high mean bacterial counts, viz. 98,800 per ml. for the Table 6 series and 73,900 per ml. for the Table 3 series (which also showed a very similar mean count of 70,900 per ml. for laboratory-pasteurized separated milk). Pasteurized separated milk from the much better supply at plant 2 showed a mean count of 22,720 per ml. (Table 4).

The flora of these pasteurized milks consisted mainly of thermoduric cocci, including a high proportion of the types previously described as characteristic of the powder flora. Coliform organisms were found in some samples (Tables 3, 4 and 6), and seven of eleven such strains isolated from plant 1 were heat-resistant.

Since the minimum satisfactory pasteurization temperature appeared to be 159° F. (for 20 sec.), it was deemed advisable to fix a working temperature of 162–163° F. to allow an adequate margin of safety. Observations showed that temporary lapses of human or automatic control could result in rapid temperature fluctuations of a more marked character than those normally observed in holder pasteurization. Moreover, the first milk pasteurized at the start of a run, or following a plant stoppage, frequently passed through the plant at too low a temperature, and failure to by-pass such milk usually resulted in contamination of the plant and high powder counts; this was allowed to happen on some occasions in order to obtain data. Another cause of low-temperature operation arose from the accumulation of film on the heating surfaces, but this was gradual and could be controlled.

Of the individual runs included in Table 2, numbers 1, 3, 4, 6, 21, 23 and 32 were made at pasteurization temperatures between 153 and 158° F.; numbers 6 and 21 represent the first milk passed through after stoppages for plant cleaning. Runs 21, 23 and 32 yielded satisfactory powder, but runs 1, 3, 4 and 6 showed extremely high bacterial counts in both pasteurized milk and powder.

It is well known that excessive heat treatment results in deleterious chemical changes which affect the solubility and (probably) the food value of milk powder. Thus Allen(1) quotes Hunziker as stating that the pasteurization and condensing of milk should not be carried out at temperatures above 160° F., but remarks that more experimental evidence is desirable concerning this point.

Since it is essential for practical purposes to establish both upper and lower temperature limits, the influence of pasteurization temperatures on the solubility and moisture content of powders was studied. Two preliminary trials were made in which the pasteurizing temperature of separated milk was gradually raised; the corresponding powder samples yielded the following solubility index values:

Pasteurizing temp. ° F.	Solubility index	
	Trial 1	Trial 1
150	0·05	0·05
152	0·05	0·05
154	0·05	0·05
156	0·10	0·10
158	0·10	0·15
160	0·15	0·15
162	0·20	0·25
163	0·30	0·30
165	0·50	0·60

Solubility index determinations of the series of 269 samples of separated milk powder (Table 8) were subject to the additional influence of possible variations of hot-air drying temperatures, but nevertheless these more extensive figures showed general confirmation of the preliminary trials. It was concluded that the solubility of powders was impaired least when pasteurizing temperatures did not exceed 159° F., whilst between 160 and 163° F. the mean solubilities showed a nearly constant value which was only slightly below that at 159° F. Between 164 and 167° F. a further reduction of solubility was apparent, but even at 167° F. the reduction was not of genuine commercial significance. It may therefore be doubted whether there is any real foundation for the belief, commonly held, that any temperature exceeding 160° F. is undesirable. Whilst the maximum heat treatment which can be given without affecting solubility is possibly rather less than 162° F. for 15 sec. (generally accepted as equivalent to holder pasteurization at 145° F. for 30 min.), it can certainly be increased to at least this figure without serious reduction of solubility. There seems no real objection, therefore, to the choice of a heat treatment which will ensure destruction of pathogenic bacteria. It may also be mentioned that the moisture content of powders was not affected by milk pasteurization temperatures (Table 8).

It is of interest that bacterial destruction and loss of solubility bears a similar relationship to pasteurization temperatures, viz. noticeable effects below 159° F. and above 164° F. Although failures of the pasteurization process result in powders of unsatisfactory bacteriological standard, the margin of safety is in reality a wide one; between the lower limit of bacterial survival and the upper limit of impaired solubility there seems to be a margin of 5–7° F., which should be ample for successful practical operation.

(3) *Passage of hot milk to the evaporator*

A supply tank for holding the hot milk is required to ensure a regular feed to the evaporator, and this also introduces a holding period for the pasteurized milk.

It was feared that prolonged holding of hot milk would introduce difficulties with thermophilic bacteria, and such trouble actually arose occasionally during the first few runs made early in 1936. A supply tank was installed of the minimum size consistent with operational safety; in addition, a system of baffles was fitted to encourage a regular flow of milk through the tank in 3–5 min.

No further trouble was experienced, apart from a few cases of enforced holding due to plant stoppages. Two such instances may be quoted when hot separated milk was held for 3 hr.; the bacterial counts increased from 80,000 to 2,320,000 and from 47,000 to 1,880,000 per ml. Nichols(3) reports cases of serious difficulty at one plant due to development of *B. calidolactis* in milk held at 160° F. for periods up to 20 hr.

Following the normal holding period of 3–5 min., slight decreases were usually observed in the bacterial counts of pasteurized milk.

(4) *The evaporation process*

For various reasons, preliminary concentration of milk precedes spray drying, but the method of evaporation varies considerably between different plants. At both plants herein described, evaporation was performed in Kestner triple effect climbing film evaporators (Fig. 1); the milk was concentrated to about 42% solids at a temperature of 110° F. This particular plant possesses certain advantages over earlier types of

Table 8. *Frequency table. Separated milk powder*

Table 9. *Frequency table. Separated milk powder*

Pasteurization temp. of milk ° F.	Log of plate count at 37° C.																				Total
	2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	7·01–7·25	7·26–7·50	
Under 158	—	—	—	—	1	—	—	1	1	2	—	—	1	1	1	3	—	2	1	—	1	15
158	1	—	—	—	—	—	—	1	—	1	1	—	1	2	—	—	—	—	—	1	—	8
159	—	—	1	—	—	—	1	4	2	3	1	3	—	—	—	—	—	—	—	1	—	16
160	—	—	—	—	2	10	7	3	3	11	12	5	5	5	2	3	—	1	—	—	—	69
161	—	—	1	—	1	5	3	6	12	8	8	5	3	3	1	—	1	—	—	—	—	57
162	—	1	1	1	2	4	5	7	7	8	5	7	5	1	—	—	—	—	—	—	—	54
163	—	—	—	—	1	1	—	3	4	5	3	5	—	—	—	1	1	—	—	—	—	24
164	1	—	—	1	—	1	2	5	—	2	1	1	—	—	—	—	—	—	—	—	—	14
165	—	—	—	—	1	—	—	1	1	2	—	—	—	—	—	—	—	—	—	—	—	5
Over 165	—	—	—	—	—	—	1	1	1	2	—	—	1	1	—	—	—	—	—	—	—	7
Total	2	1	3	2	8	21	19	32	31	44	31	26	16	13	4	7	2	3	1	2	1	269

Table 10. *Influence of hot-air temperature on quality of separated milk powder*

Hot-air inlet temp. ° C.	No. of samples	Mean log plate count at 37° C.	Corresponding actual plate count	Range of counts		Coliform-positive samples		Spore-forming anaerobes, positive samples		Mould-positive samples			Mean moisture %	Mean solubility index
				Min.	Max.	No.	%	No.	%	No.	%	Mean count		
145–149	9	4·79822	62,840	29,000	184,000	7	77·7	2	22·2	3	33·3	60	4·87	0·13
150–154	14	4·57992	38,010	3,100	1,238,000	8	57·1	4	28·6	3	21·4	27	3·83	0·27
155–159	18	4·63708	43,360	3,700	510,000	5	27·7	5	27·8	5	27·7	20	3·86	0·64
160–161	25	4·62596	42,270	200	7,750,000	9	36·0	3	12·0	3	12·0	20	4·40	0·48
162	17	4·74761	55,930	200	640,000	5	29·4	3	17·6	1	5·9	10	4·22	0·15
163	21	4·82204	66,370	4,200	3,760,000	4	19·0	4	19·0	Nil	Nil	Nil	4·03	0·42
164	25	4·72995	53,690	1,500	12,150,000	6	24·0	4	16·0	2	8·0	40	3·90	0·28
165	40	4·60455	40,230	2,700	313,400	6	15·0	7	17·5	4	10·0	13	4·07	0·39
166	30	4·13424	13,260	400	517,000	3	10·0	2	6·7	3	10·0	33	3·83	0·36
167	16	4·19724	15,750	3,300	130,000	Nil	Nil	2	12·5	1	6·25	10	3·64	0·53
168	15	4·48489	30,540	2,700	358,000	Nil	Nil	3	20·0	Nil	Nil	Nil	4·06	0·27
169–170	6	4·13133	13,530	1,000	91,200	Nil	Nil	2	33·3	Nil	Nil	Nil	3·91	0·51
Tota	236	4·52964	33,860	200	12,150,000	53	22·5	41	17·4	25	10·6	27	4·03	0·38

evaporators, and by virtue of continuous operation is well suited for combination with spray-drying plants.

The authors are unaware of any published bacteriological information dealing with this method of evaporation, due no doubt to the small number of plants in operation.

The bacteriological problems introduced probably involved several conflicting factors, viz. (*a*) destruction of bacteria due to increasing osmotic pressure, (*b*) addition of bacteria by contamination from surfaces, and (*c*) growth of some species at the low temperatures employed. In some cases evaporation was accompanied by decreased numbers of bacteria, but more frequently a considerable increase was found, even after allowance had been made for increased concentration of the liquid.

Thus the mean bacterial count of evaporated separated milk was 518,000 per ml. (ranging from 35,000 to 81,600,000) at plant 1 (*A*, Fig. 1), and 1,237,000 per ml. (ranging from 52,000 to 11,450,000) at plant 2. Since the primary source of bacteria must be the flora of the pasteurized milk passed into the evaporator, a close relationship was expected, and twenty-eight pairs of corresponding samples (Table 5) showed a correlation coefficient of 0·67. Of the fourteen highest evaporated milk counts obtained from plant runs (Table 2), nine corresponded to pasteurized milk counts exceeding 200,000 per ml., but two were derived from pasteurized milks between 50,000 and 100,000 per ml. and three from pasteurized milks having counts below 50,000 per ml. Conversely, the twelve lowest evaporated milk counts showed corresponding pasteurized milk counts under 50,000 per ml. in six cases, between 50,000 and 200,000 per ml. in three cases, and over 200,000 per ml. in three cases.

Other investigations made in these laboratories have shown that milk concentrated in a vacuum pan by the usual batch condensing process (as in the manufacture of tinned evaporated milk) generally shows a decreasing bacterial count as evaporation proceeds. Concentration by the batch process, however, requires an evaporation period of several hours, whereas continuous evaporation in a climbing film evaporator occupies only a few minutes at a low temperature (110° F.).

Destruction of bacteria during the continuous evaporation process did not normally occur, but probably took place during some runs, e.g. nos. 11, 20, 27, 29, and 32 (Table 2).

It was clear from the evaporated milk flora that considerable contamination frequently occurred during evaporation. This flora naturally consisted partly of thermoduric cocci derived from the pasteurized milk, but general miscellaneous contamination was often evident. The most striking feature was undoubtedly the common occurrence of coliform organisms, which were present in 75·8% of evaporated separated milk samples from plant 1 and 87·9% from plant 2. Of thirty-four coliform strains isolated from evaporator samples, twenty (i.e. 58·8%) were heat-resistant.

Evaporator contamination arose from the combined effects of surface contamination and bacterial growth, both of which were encouraged by the construction of the plant and its conditions of operation. Evaporation was effected by continuous passage of a thin climbing film of liquid through three sections of calandria tubes, each of these effects consisting of a nest of tubes approximately 30 ft. long and 2 to 3 in. diameter (Fig. 1). Owing to the lengthy runs entailed, film accumulated on the plant surfaces. The cleansing and sterilization of the plant itself was by no means a simple operation, apart from the

necessity of removing milk deposits. It was regularly observed that perfunctory cleansing of the evaporator was followed by high counts and large numbers of coliform organisms in the evaporated milk.

The accumulation of milk deposits, especially in situations difficult of access for cleaning purposes, provided favourable conditions for contamination of the plant and for the multiplication of species capable of growth at 110° F. Sherman & Wing(12) recorded the optimum growth temperature of *Str. durans* as 50° C. (122° F.).

Under such circumstances, the thermoduric species surviving pasteurization were readily multiplied during the evaporation process; these species constituted the main flora of spray-dried powders. In addition, the large size and general construction of the evaporator encouraged inadequate cleansing, and the time required for efficient cleaning sometimes presented a choice between very long evaporating runs or more frequent stops for rapid superficial cleaning. (On occasion film formation on calandria tubes resulted in enforced cleaning intervals.)

The bacterial count of evaporated milk therefore tended to increase during long runs, although this was not invariable and happened less frequently when the plant was very thoroughly cleansed before commencing the run. One good example of such an increase is shown below; in this case *Str. thermophilus* was the predominant species.

Time from start of run	Evaporated milk			Yeast and mould count per ml.	Powder		Mould count per g.
	Plate count per ml. 37° C.		Coliform organisms		Plate count per g. 37° C.	Coliform organisms	
4	808,000	+	0·1 ml.	1	87,000	Negative	Nil
6	869,000	+	0·1 ml.	2	82,000	,,	30
8	935,000	+	0·1 ml.	Nil	29,000	,,	Nil
10	3,230,000	+	0·1 ml.	Nil	1,620,000	,,	Nil
12	18,800,000	+	0·01 ml.	10	12,000,000	,,	10

The data in Table 2 show, however, that it was quite possible to obtain evaporated milks of low bacterial content and free from coliform contamination. Such results could be achieved by adopting a satisfactory plant-cleaning treatment, which comprised the use of very strong detergent solutions and employing the longest practicable soaking period, combined with the use of revolving power-driven wire brushes arranged to pass up and down the complete length of the calandria tubes. False economy in the use of detergent must be avoided; for satisfactory bacteriological results a 2% solution of a detergent powder consisting of 90% caustic soda was found necessary.

A favourable feature of the climbing film method was the almost complete absence of yeast and mould contamination.

The bacterial counts of the evaporated milk undoubtedly influenced the bacterial counts of the spray-dried powder, which would be expected in view of the evaporated milk flora; twenty-seven pairs of corresponding samples yielded a correlation coefficient of 0·68. The diagram in Fig. 2 illustrates the general relationship between the plate counts of pasteurized milk, evaporated milk, and milk powder. The importance of evaporator contamination was also shown by comparing plants 1 and 2 (Tables 3 and 4). Plant 2 handled the better raw-milk supply, accompanied by lower mean pasteurized milk counts, but as a result of greater evaporator contamination it produced powders of higher mean bacterial count.

Nevertheless, the relationship was not complete. Thus the fourteen highest evaporator counts already cited corresponded to powder counts distributed as follows:

Over 500,000	10 samples
100,000–500,000	1 sample
50,000–100,000	1 sample
10,000– 50,000	2 samples

Again, the twelve lowest evaporator counts yielded powder counts:

Over 500,000	1 sample
100,000–500,000	4 samples
50,000–100,000	1 sample
10,000– 50,000	3 samples
Under 10,000	3 samples

(5) *Passage of evaporated milk to the atomizer*

Supply tanks were required for feeding the concentrated milk via the atomizer to the drying chamber. Some storage of evaporated milk at this stage was needed to cover temporary stoppages of other sections of the plant, but prolonged holding at the temperature of discharge from the evaporator would obviously encourage multiplication of bacteria. Two tanks were therefore employed to ensure a rotational atomizer feed.

Furthermore, transfer of concentrated milk from evaporator discharge to atomizer feed tank required a pump and long pipe lines, a dangerous source of contamination inseparable from the design and lay-out of the particular plants (Fig. 1). The data given in Tables 2–4 show that such contamination was sometimes considerable, and to avoid it strict attention to plant hygiene was needed.

Separated milk samples taken from the atomizer feed tank (*B*, Fig. 1) had a mean plate count of 2,799,000 per ml. (ranging from 33,800 to 137,000,000 at plant 1, and a mean of 1,770,000 per ml. (ranging from 95,000 to 39,000,000) at plant 2. Coliform contamination also was more pronounced than in samples taken direct from the evaporator. Plate counts of samples from the atomizer feed tanks were naturally related to those of samples direct from the evaporator; a correlation coefficient of 0·81 was obtained from twenty pairs.

Contamination of a more general nature was even more noticeable in the concentrated milk fed to the atomizer. This was due partly to infection from the surfaces of pump, pipe lines, and storage tanks, and partly to atmospheric contamination of the concentrated milk held in the storage tanks. In addition, rapid multiplication of bacteria during the run was to some extent inevitable owing to the continued presence of milk standing at a favourable temperature. Plant samples showed that in general contamination from pipe lines was less serious than the increases which occurred during storage.

In addition to thermoduric streptococci, various micrococci were found; some of these were non-thermoduric. Of twenty-four coliform strains examined, twelve proved to be heat resistant. Small numbers of yeasts were also found quite frequently.

Some of the plant runs (Table 2) showed clearly, however, that it was possible to maintain the numbers of bacteria at a reasonably low level, although very difficult to achieve complete freedom from coliform contamination.

In common with pasteurizer and evaporator samples, the plate counts of concentrated milks entering the dryer were significantly related to plate counts of powders ($r = 0{\cdot}77$ for nineteen pairs). A high plate count at the atomizer feed was commonly followed by a high powder count, e.g. runs 1, 4, 5, 6, 9, 10, 11, 13 and 43 (Table 2). In other cases,

however, the drying process destroyed very large numbers of the bacteria present in the atomizer feed and low powder counts resulted, e.g. runs 23, 26, 28, 32 and 46. Run 21 was anomalous in that high bacterial counts at each stage preceding drying were followed by a low powder count. Examination of the seventeen highest atomizer feed plate counts revealed the following distribution of powder counts:

Over 1,000,000	5 samples
500,000–1,000,000	1 sample
100,000– 500,000	5 samples
50,000– 100,000	1 sample
10,000– 50,000	4 samples
Under 10,000	1 sample

The results may be explained by the presence in the atomizer feed of varying numbers of miscellaneous non-heat-resistant organisms, many of which were destroyed by drying.

(6) *The drying process*

The hot-air drying process results in very considerable destruction of bacteria, the extent of which is shown by the summarized data presented in Tables 3 and 4. Moreover, it must be remembered that these data include runs wherein serious faults were known to exist, a circumstance which accounted for the mean count of 109,400 per g. for separated milk-powder runs at plant 1 (Table 3) as against a mean of 57,790 per g. for all such samples from this plant (Table 1).

Philip(4) stresses the importance of employing the highest possible temperature for the inlet air (i.e. the current of hot air meeting the mist produced by the atomizer), in order to obtain high thermal efficiency. The susceptibility of dairy products to heat changes must, however, impose limitations, and there is always the risk that some particles may be prematurely dried and consequently subjected to the heating effect of air above the final exit air temperature. Scott(5) points out that the use of high air temperatures requires a high relative velocity between the particles and the air; where tranquil air streams are used, low air temperatures (below 130° C.) are essential to avoid overheating. In practice the temperature of the hot-air current probably varies from 130 to 170° C. between different types of spray driers, whilst the time of exposure to hot-air drying is similarly variable.

The two Kestner plants normally employed a hot-air inlet temperature between 160 and 166° C., whilst the exit air left the plant at about 90° C. These plants were based on the principle of very rapid passage (a few seconds) of the particles through the hot drying zone, and in addition the powder was rapidly removed from the conical base of the drying chamber (*C* and *D*, Fig. 1), so that the process might be termed a 'high temperature-short time' one.

The effects of heat treatment applied during the drying process were investigated in the same manner as the effects of pasteurizing temperatures. A series of 236 separated milk powders from plant 1, produced at hot-air inlet temperatures between 145 and 170° C., yielded the data presented in Tables 10 and 11.

No very pronounced variations in the powder plate counts were obvious within the temperature ranges studied. The frequency distributions indicated a tendency towards higher counts at hot-air temperatures below 159° C. and lower counts above 165° C.; the mean counts also showed a decrease at 166–170° C., whilst the ranges of variation became smaller at 165° C. and above. The coliform test yielded much more striking

results, ranging from 77·7% positive samples below 149° C. to complete absence of positives from 167 to 170° C. The incidence of positive samples appeared to be noticeably increased below 155° C. and decreased above 164° C.

It was concluded that a fairly clear upper zone of increased bacterial destruction commenced at about 165° C., but the lower boundary of increasing survival appeared to be less definite, although temperatures below 155° C. were clearly undesirable owing to pronounced survival of coliform organisms.

The occurrence of spore-forming anaerobes seemed to be unrelated to hot-air drying temperatures. The number of mould-positive samples was small, but there appeared to be a tendency towards survival at low temperature ranges and destruction at the highest temperatures.

Somewhat surprisingly, the moisture content of powders showed no definite relationship to hot-air temperatures, apart from increased moisture content below 150° C. Similarly, the solubility of powders was slightly superior when dried below 155° C., but air inlet temperatures up to 170° C. produced no significant deterioration in solubility.

To sum up, there was clearly a very wide margin of safety for successful commercial operation.

The total heat treatment was divided between two processes, viz. pasteurization and drying (the condensing temperatures were very low at the particular plants) and the records were therefore searched for samples illustrating the combined effects of both processes. By selecting all powder samples combining low pasteurizing temperature with low air temperature, and high pasteurizing temperature followed by high air temperature, the following comparisons were obtained.

Combined effects of pasteurization and hot-air temperatures at extreme ranges

	Pasteurization temp. of skim milk 159° F. or lower. Hot-air inlet temp. 162° C. or lower	Pasteurization temp. of skim milk 163° F. or higher. Hot-air inlet temp. 166° C. or higher
No. of skim milk powder samples	15	13
Mean log plate count of powder	4·83340	4·39803
Corresponding actual count	68,140	25,000
Range of counts	10,500–7,750,000	2,700–115,000
Coliform positive samples	8 (53·3%)	Nil
Spore-forming anaerobes positive	3	2
Mould positive samples	2	1
Mean moisture per cent	4·37	3·96
Mean solubility index	0·21	0·35

The number of such occurrences was not large, but the differences between the two groups were obviously significant. The low-temperature group showed a much wider range of plate counts, a somewhat higher mean plate count, a striking survival of coliform organisms as against complete destruction in the high-temperature group, slightly higher moisture content and slightly superior solubility.

It therefore seemed clear that parallel variations from normal heat treatment in the two processes might have a pronounced influence upon the quality of powder; equally it might be inferred that contrary variations would tend to neutralize one another.

It may be mentioned that Wright(13) considers that protein insolubility due to denaturation in solution is quite distinct from insolubility caused by heating of powder in a dry state.

Finally, the influence of the time of exposure to hot-air treatment must be considered.

Table 11. *Frequency table. Separated milk powder*

Table 11. *Frequency table. Separated milk powder*

Hot-air inlet temp. ° C.	Log of plate count at 37° C.																		
	2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	Total
145–149	—	—	—	—	—	—	—	—	1	5	—	2	1	—	—	—	—	—	—	9
150–154	—	—	—	—	1	1	1	2	2	3	1	1	—	—	—	2	—	—	—	14
155–159	—	—	—	—	—	2	—	4	—	4	3	2	1	2	—	—	—	—	—	18
160	—	—	—	—	—	1	—	1	2	3	—	—	2	1	—	—	—	—	1	11
161	1	—	—	—	—	1	—	2	2	3	3	2	—	—	—	—	—	—	—	14
162	1	—	—	1	—	1	—	—	1	4	1	2	2	3	1	—	—	—	—	17
163	—	—	—	—	—	2	1	3	2	1	5	2	1	2	—	—	1	1	—	21
164	—	—	—	1	1	1	2	2	2	7	3	1	—	1	1	2	—	—	1	25
165	—	—	—	—	1	3	2	5	7	5	5	9	3	—	—	—	—	—	—	40
166	—	1	1	—	2	2	6	3	3	2	5	1	1	3	—	—	—	—	—	30
167	—	—	—	—	—	4	2	2	4	2	1	1	—	—	—	—	—	—	—	1(
168	—	—	—	—	1	1	1	2	2	2	3	—	2	1	—	—	—	—	—	15
169–170	—	—	1	—	—	1	1	—	1	1	1	—	—	—	—	—	—	—	—	6
Total	2	1	2	2	6	20	16	26	29	42	31	23	13	13	2	4	1	1	2	236

Table 12. *Corresponding samples of separated milk powder taken from main dryer and bag outlets*

Plate count of bag-powder as percentage of main dryer count, 54 pairs		Results of other bacterio-logical tests	Main-dryer positive Bag-powder positive		Main-dryer negative Bag-powder negative		Main-dryer positive Bag-powder negative		Main-dryer negative Bag-powder positive		Mean moisture %, 54 pairs		Mean solubility index, 55 pairs	
%	No. of pairs		No. of pairs	% of pairs	No. of pairs	% of pairs	No. of pairs	% of pairs	No. of pairs	% of pairs	Main dryer	Bags	Main dryer	Bags
Under 10·0	3	Coliform organisms	11	20·4	31	57·4	11	20·4	1	1·8	4·6	2·4	0·40	1·22
10·0–19·9	5	54 pairs	—	—	—	—	—	—	—	—				
20·0–29·9	9	Anaerobes	6	10·9	41	74·5	5	9·1	3	5·5				
30·0–39·9	4	55 pairs	—	—	—	—	—	—	—	—				
40·0–49·9	7	Moulds	—	—	—	—	—	—	—	—				
50·0–59·9	4	55 pairs	3	5·5	44	80·0	5	9·1	3	5·5				
60·0–69·9	5													
70·0–79·9	7													
80·0–89·9	1													
90·0–99·9	Nil													
Bag count higher than main dryer count	9													

As has been stated, this exposure is very short in the Kestner process, but a small proportion of powder particles (about 1–2% of the output) passes out with the exit air and must be recovered by means of bag filters (*E*, Fig. 1). This 'bag' powder may be exposed for a long period to the exit air current at 180–190° F. In some spray-drying plants much of the powder output must be recovered in this way, whilst in others no particular attention is devoted to the speedy removal of powder from the drying chamber.

The data summarized in Table 12 were obtained from fifty-four pairs of samples taken from the main powder outlet and the bag filters; the samples were taken to correspond with one another as nearly as possible. Usually the plate counts of bag-filter powder were considerably lower than those of powder discharged direct from the dryer, e.g. twenty-eight of fifty-four bag-powder counts were under 50% of the main dryer counts. Similarly, the bag-filter powders showed a much reduced incidence of coliform organisms and a slightly reduced incidence of spore-forming anaerobes and moulds. This increased bacterial destruction was accompanied by significant chemical changes; the moisture content of bag-filter powders was low, whilst the solubility was inferior, although still greatly superior to that of roller-dried powder.

The time of exposure to hot-air drying was probably a more important factor than the actual temperature employed, particularly when the production of highly soluble powders was desired. Scott(5), quoting Wright, also states that exposure to dry heat may result in considerable decrease in solubility.

It seems likely that different methods of atomization (e.g. centrifugal and pressure-jet atomizers) might lead to variations in hot-air treatment which would influence the degree of bacterial destruction, but no opportunity arose to investigate this point.

For the sake of completeness the relationship of bacteriological quality to moisture content (340 samples) and solubility (305 samples) was tabulated from the records (Tables 13–16). Bacteriological quality appeared to be unrelated to moisture content or solubility, except possibly at extreme values (due no doubt to a common relationship to the effects of extremes of heat treatment). There was, however, a clear relationship between moisture content and solubility (Table 13).

(7) *Coliform types from plant samples*

Detailed confirmatory tests were made of 'presumptive positives' obtained from thirty plant runs. In these runs, twenty-one pasteurizer samples, five evaporator samples, no atomizer feed samples,* and nineteen powder samples were coliform negative. (False positives have been excluded.) The positive samples yielded the following coliform types:

	Pasteurizer		Evaporator		Atomizer feed		Powder	
	Total no.	No. heat resistant	Total no.	No. heat resistant	Total no.	No. heat resistant	Total no.	No. heat resistant
Coli I	8	5	26	17	18	9	7	3
Coli II	1	1	—	—	—	—	1	1
Intermediate I	—	—	—	—	1	Nil	—	—
Intermediate II	—	—	2	2	1	1	—	—
Aerogenes I	2	1	5	1	4	2	5	2
Irregular V	—	—	—	—	—	—	1	Nil
Irregular VIII	—	—	1	Nil	—	—	—	—
Cloacae	—	—	—	—	—	—	1	Nil
Total	11	7	34	20	24	12	15	6

* Ten of the presumptive positives were not confirmed in detail.

Table 13. *Relation between bacteriological quality and moisture content of separated milk powder*

Moisture %	No. of samples	Mean log plate count at 37° C.	Corresponding actual plate count	Mould-positive samples			Mean solubility index
				No.	%	Mean count	
1·3–2·4	10	4·61521	41,230	1	10·0	20	0·87
2·5–2·9	24	5·09688	125,000	4	16·6	18	0·42
3·0–3·4	45	4·64987	44,654	4	8·8	13	0·48
3·5–3·9	72	4·84496	69,978	6	8·3	18	0·40
4·0–4·4	80	4·65456	45,140	9	11·2	26	0·32
4·5–4·9	57	4·74228	55,242	6	10·5	45	0·23
5·0–5·4	31	4·83593	68,533	3	9·7	13	0·21
5·5–7·1	21	4·71209	51,535	2	9·5	20	0·17
All samples	340	4·75912	57,427	35	10·3	24	0·35

Table 14. *Frequency table of plate count and moisture content of separated milk powder*

Moisture %	Log of plate count at 37° C.																				
	2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	7·01–7·25	7·26–7·50	Total
1·3–2·4	—	—	1	—	—	—	—	4	—	1	1	—	1	1	—	1	—	—	—	—	—	10
2·5–2·9	—	—	—	—	—	1	1	2	4	3	3	3	—	1	—	2	1	1	—	2	—	24
3·0–3·4	—	—	—	—	—	3	6	3	5	11	4	7	—	3	2	1	—	—	—	—	—	45
3·5–3·9	1	—	1	—	1	6	5	7	7	9	7	1	5	9	6	2	2	1	1	1	—	72
4·0–4·4	—	—	—	1	1	5	6	11	10	14	10	6	9	2	—	3	1	—	—	—	1	80
4·5–4·9	1	—	—	—	1	2	3	6	13	6	10	3	3	5	1	3	—	—	—	—	—	57
5·0–5·4	—	—	—	—	—	2	2	3	4	3	3	7	2	4	—	—	—	1	—	—	—	31
5·5–7·1	—	—	—	—	1	2	—	1	1	4	6	1	1	1	1	1	1	—	—	—	—	21
Total	2	—	2	1	4	21	23	37	44	51	44	28	21	26	10	13	5	3	1	3	1	340

Table 15. *Relation between bacteriological quality and solubility of separated milk powder*

Solubility index	No. of samples	Mean log plate count at 37° C.	Corresponding actual plate count	Mould positive no.
0·05	42	4·83072	67,721	7
0·10	82	4·68279	48,172	10
0·15	20	5·17751	150,480	2
0·2	66	4·87610	75,179	12
0·3	30	4·53631	34,380	2
0·4, 0·5, 0·6	22	4·87435	74,877	2
0·7, 0·8, 0·9	16	4·40733	25,546	Nil
1·0–4·8	27	4·54809	35,326	4
All samples	305	4·75047	56,296	39

Table 16. *Frequency table of plate count and solubility of separated milk powder*

Solubility index	Log plate count at 37° C.																				
	2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	7·01–7·25	7·26–7·50	Total
0·05	—	—	—	—	—	5	2	3	4	6	5	6	3	5	1	1	1	—	—	—	—	42
0·10	—	—	—	1	1	7	3	14	13	6	15	5	4	5	1	5	1	1	—	—	—	82
0·15	—	—	—	—	—	1	—	—	2	3	2	2	2	4	2	2	—	—	—	—	—	20
0·2	—	—	—	—	—	2	4	7	9	12	8	6	6	6	2	—	1	1	—	—	2	66
0·3	—	—	1	—	—	3	4	4	3	5	2	2	1	2	1	—	1	1	—	—	—	30
0·4–0·6	—	—	—	—	—	2	1	1	2	4	3	4	1	2	—	1	—	—	1	—	—	22
0·7–0·9	—	—	—	—	1	—	3	3	2	3	1	2	1	—	—	—	—	—	—	—	—	16
1·0–2·8	—	—	2	—	1	3	4	1	2	2	4	4	—	1	1	2	—	—	—	—	—	27
Total	—	—	3	1	3	23	21	33	37	41	40	31	18	25	8	11	4	3	1	—	2	305

Heat-resistant strains therefore comprised 63·6% of the pasteurizer strains, 58·8% of evaporator strains, 50·0% of atomizer feed strains, and 40·0% of powder strains (figures previously quoted showed 48·5% heat-resistant strains from all powder samples). These figures support the previous conclusions that heat-resistant species tend to predominate in the pasteurized milk flora, but some recontamination by more varied types occurs during the evaporator stages. Moreover, it appeared possible that the effects of pasteurization and drying were not necessarily similar, since non-heat-resistant strains occurred more frequently among powder strains. It was also observed that *coli* I comprised fifty-two of sixty-nine strains (75·4%) obtained from the pasteurizer and evaporator stages, but only seven of fifteen strains (46·6%) from powder samples. This finding, in conjunction with the coliform data already given for all powder samples, suggests that *coli* I is less resistant to drying than the *aerogenes* and *cloacae* types.

(8) *Variations during a run*

It has previously been recorded(2) that a variable increase in the bacterial content of powder tends to occur as the run progresses; Nichols(3) obtained somewhat irregular results but confirmed the same general indications. The increase is doubtless due to several factors which have already been discussed, viz. multiplication of thermoduric and thermophilic bacteria during storage of the raw milk, in the plant itself, and in evaporated milk held in the atomizer feed tanks, combined with the accumulation of film on plant surfaces.

Study of similar bacterial increases which occur in milk pasteurizing plants has resulted in the adoption by most commercial operators of a limited length of run and of intermittent sterilization of the plant. Similar information concerning the spray-drying process is obviously a matter of practical importance.

The following nine runs displayed fairly regular increases in the bacterial content of the powders, and have been chosen because the pasteurizing and drying temperatures remained practically constant throughout.

	Period in run							
	2 hr.	4 hr.	6 hr.	8 hr.	10 hr.	12 hr.	14 hr.	16 hr.
1 Powder count	29,000	7,400	19,500	1,520,000	149,000	2,460,000	2,410,000	780,000
Coliforms	−	+	−	−	−	−	+	−
2 Powder count	10,200	7,500		44,900	76,600			
Coliforms	−	−		−	−			
3 Powder count				137,200	89,000	75,000	490,000	1,760,000
Coliforms				−	−	−	−	−
4 Powder count	264,000	796,000	11,900,000	14,000	62,000	120,000	760,000	
Coliforms	−	−	−	−	−	−	+	
5 Powder count	700	368,000	9,700	5,900	7,700			
Coliforms	−	−	−	−	−			
6 Powder count	11,100	7,900		254,000	320,000	510,000	1,150,000	19,500,000
Coliforms	−	−		−	−	−	−	+
7 Powder count	15,800	19,700	71,700	95,100		63,200		297,000
Coliforms	−	−	−	−		+		+
8 Powder count		190,000	407,000	231,000		1,560,000		
Coliforms		−	+	−		−		
9 Powder count	57,000	87,000	82,300	29,000	1,620,000	12,900,000		
Coliforms	−	−	−	−	−	−		

Intermittent cleansing and sterilization (indicated by the dotted line) took place from 7 to 9 hr. after commencement of the run; although this procedure was often somewhat

hurried, it was clearly of value, e.g. in runs 1, 3, 4 and 7. Such a stoppage would obviously have been desirable after about 6–7 hr. during some runs, e.g. in runs 1, 4 and 8. Coliform organisms appeared to occur most frequently towards the end of a long run.

General observations of a large number of runs were by no means so consistent as in these selected cases. The basic causes of variation were not constant for every run and much seemed to depend upon the initial bacteriological cleanliness of the plant.

Summarized data for 333 samples taken at various periods between the start and 23 hr. of operation are given in Tables 17 and 18. These more general and extensive figures show little variation of either the mean counts or the frequency distributions for periods up to 9 hr. after starting the run. For runs exceeding 10 hr. duration, however, both the mean plate counts and the range of distribution show significant increases. The incidence of coliform species was lowest during the first 2 hr. of operation, and was noticeably increased beyond 10 hr., but no consistent results were observed in the incidence of spore-forming anaerobes or moulds.

The mean moisture content remained fairly constant throughout, but the solubility of powders appeared to deteriorate after about 8–9 hr.

These figures refer to plant 1 (poor milk supply), but similar findings were obtained at plant 2 (good milk supply). It was concluded, therefore, that an unbroken run should never exceed 9 hr. duration, and it would probably be safer to fix a limit of 7 hr.

A further problem arises in connexion with storage of milk supplies overnight, which may sometimes be necessary to provide a supply for commencing operations before fresh milk arrives. At some plants evaporated milk is held overnight for this purpose, which requires only small storage capacity, but introduces bacteriological problems, as Nichols(3) also observed. From the foregoing discussions it is clear that this procedure is potentially dangerous. The evaporated milk flora contains a high proportion of specialized thermoduric species which constitute the main powder flora, whilst inadequate cooling results in further multiplication of these organisms. Storage of raw milk overnight at a temperature not exceeding 40° F. was found entirely satisfactory. Powders produced from such cold-stored milk had uniformly low plate counts, in spite of the poor supply handled at one plant.

(9) *Seasonal variations*

Nichols(3) has observed that seasonal variations in quality may occur when the general bacterial standard is normally poor, but are not observed at plants producing powder of uniformly good quality.

Summer operation introduces special difficulties, since deterioration of the raw-milk supply and high atmospheric temperatures coincide with the peak period of operation. It was expected that such difficulties would arise at plant 1, but in practice no very pronounced differences were observed between winter and summer operation. The data obtained from 411 samples of separated milk powder produced by this plant are classified on a monthly basis in Tables 19 and 20. The data for December are only of limited value since very few samples were obtained.

The mean plate count over the whole winter period (1 October to 31 March) was 50,110, compared with a mean of 61,420 for the summer period; the mean of all samples was 57,790 per g. The range of plate counts was, however, much wider during the summer period; this is very clearly shown by the frequency distributions. Of thirty-two plate

Table 17. *Variations in quality of separated milk powder throughout the run*

Time from start of run hr.	No. of samples	Mean log plate count at 37° C.	Corresponding actual plate count	Range of counts		Coliform-positive samples		Spore-forming anaerobes, positive samples		Mould positive samples			Mean moisture %	Mean solubility index
				Min.	Max.	No.	%	No.	%	No.	%	Mean count		
Start	18	4·53963	34,640	3,400	796,000	3	16·7	3	16·7	4	22·2	50	4·40	0·22
1	13	4·27682	18,920	1,100	1,190,000	1	7·7	Nil	Nil	1	7·7	10	4·14	0·20
2	32	4·70184	50,330	5,500	640,000	3	9·4	5	15·6	3	9·4	20	4·03	0·39
3	32	4·71261	51,600	5,700	12,000,000	8	25·0	6	18·75	3	9·4	20	3·95	0·26
4	25	4·58302	38,280	3,500	2,070,000	9	36·0	4	16·0	3	12·0	13	4·12	0·24
5	29	4·63815	43,470	1,500	13,000,000	5	17·2	7	24·1	6	20·7	10	3·96	0·17
6	87	4·64132	43,780	200	7,700,000	26	29·9	9	10·3	9	10·3	34	3·92	0·39
7	65	4·52706	33,660	1,000	2,470,000	13	20·0	6	9·2	7	10·8	22	4·09	0·35
8–9	14	4·73236	54,000	3,100	1,560,000	4	28·6	3	21·4	1	7·1	50	4·47	0·51
10–23	18	5·55542	359,270	11,000	19,500,000	7	38·8	3	16·7	Nil	Nil	Nil	3·96	0·48
Totals	333	4·66054	45,770	200	19,500,000	79	23·7	46	13·8	37	11·1	26	4·07	0·33

Table 18. *Frequency table of hourly variations throughout the day of separated milk powder*

Time from start of run hr.	Log of plate count at 37° C.																				Total samples
	2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	7·01–7·25	7·26–7·50	
Start	—	—	—	—	—	3	1	2	4	2	1	2	1	1	1	—	—	—	—	—	—	18
1	—	—	—	1	—	2	1	3	2	2	—	1	—	—	—	1	—	—	—	—	—	13
2	—	—	—	—	—	1	4	4	5	5	1	4	5	2	1	—	—	—	—	—	—	32
3	—	—	—	—	—	1	4	5	4	7	4	3	—	1	1	—	—	—	—	2	—	32
4	—	—	—	—	—	4	2	4	3	3	3	2	1	—	—	2	1	—	—	—	—	25
5	—	—	—	1	1	2	2	1	3	6	5	4	3	—	—	—	—	—	—	1	—	29
6	2	1	1	1	—	4	3	10	9	16	13	13	6	5	—	1	—	1	1	—	—	87
7	—	—	—	1	4	7	7	10	4	8	8	5	2	5	1	2	1	—	—	—	—	65
8–9	—	—	—	—	1	1	2	—	3	—	3	—	—	—	1	2	—	—	—	—	—	14
10–23	—	—	1	—	—	—	—	2	1	—	—	1	2	1	2	5	2	—	—	1	—	18
Total	2	1	2	4	6	25	26	41	38	49	38	35	21	15	7	13	4	1	1	4	—	333

Table 19. *Seasonal variations in quality of separated milk powder*

Month	No. of samples	Mean temp. Pasteurization ° F.	Mean temp. Hot air ° C.	Mean log plate count at 37° C.	Corresponding actual plate count	Range of counts Min.	Range of counts Max.	Coliform-positive samples No.	Coliform-positive samples %	Spore-forming anaerobes No.	Spore-forming anaerobes %	Yeast- and mould-positive samples No.	Yeast- and mould-positive samples %	Yeast- and mould-positive samples Mean count
Jan.	21	160	157	4·76898	58,750	5,600	510,000	12	57·1	6	28·6	4	19·0	28
Feb.	19	159	159	4·62263	41,940	6,000	410,000	13	68·4	6	31·6	5	26·3	12
Mar.	11	160	163	4·81138	64,770	3,600	640,000	5	45·4	1	9·1	3	27·3	20
Apr.	18	161	165	4·01602	10,370	1,000	308,000	Nil	Nil	1	5·5	1	5·5	40
May	48	162	164	4·95973	91,140	1,100	2,470,000	11	22·9	6	12·5	10	20·8	26
June	50	162	163	4·96927	93,170	3,400	19,500,000	11	22·0	4	8·0	6	12·0	23
July	50	161	164	4·74318	55,360	400	18,500,000	10	20·0	10	20·0	2	4·0	30
Aug.	68	161	164	4·77936	60,170	200	11,900,000	8	11·8	8	11·8	3	4·4	10
Sept.	54	161	165	4·77894	60,100	3,500	12,900,000	4	7·4	5	9·3	9	16·6	35
Oct.	32	162	164	4·58347	38,320	4,900	375,000	5	15·1	1	3·0	4	12·1	10
Nov.	32	161	160	4·67289	47,090	3,700	1,240,000	12	37·5	6	18·7	6	18·7	18
Dec.	8	161	152	5·12279	132,680	3,100	3,760,000	5	62·5	3	37·5	2	25·0	85
Totals	411			4·76187	57,790	200	19,500,000	96	23·2	57	13·8	55	13·4	25

Table 20. *Frequency table of seasonal variations throughout the year of separated milk powder*

Month	Log of plate count at 37° C. 2·26–2·50	2·51–2·75	2·76–3·00	3·01–3·25	3·26–3·50	3·51–3·75	3·76–4·00	4·01–4·25	4·26–4·50	4·51–4·75	4·76–5·00	5·01–5·25	5·26–5·50	5·51–5·75	5·76–6·00	6·01–6·25	6·26–6·50	6·51–6·75	6·76–7·00	7·01–7·25	7·26–7·50	Totals	
Oct.	—	—	—	—	—	3	3	3	5	7	4	1	5	1	—	—	—	—	—	—	—	32	
Nov.	—	—	—	—	—	1	2	6	2	8	5	4	3	—	—	1	—	—	—	—	—	32	
Dec.	—	—	—	—	1	—	—	—	1	2	—	1	—	—	1	1	—	1	—	—	—	8	(1)
Jan.	—	—	—	—	—	2	—	2	2	4	3	4	2	2	—	—	—	—	—	—	—	21	
Feb.	—	—	—	—	—	—	2	2	4	2	6	—	2	1	—	—	—	—	—	—	—	19	
Mar.	—	—	—	—	—	2	—	1	3	—	—	—	1	2	2	—	—	—	—	—	—	11	
Apr.	—	—	1	—	3	4	2	3	—	3	—	1	1	—	—	—	—	—	—	—	—	18	
May	—	—	—	1	—	3	3	4	5	5	3	7	4	5	3	1	3	—	—	—	1	48	
June	—	—	—	—	—	2	3	5	4	7	10	3	4	3	2	5	1	—	—	—	1	50	
July	—	1	1	1	1	2	4	3	5	3	8	6	3	6	1	1	1	—	1	1	1	50	(2)
Aug.	2	—	1	1	2	1	5	5	8	11	6	7	6	4	2	2	—	3	1	1	—	68	
Sept.	—	—	—	—	—	4	3	11	4	8	8	5	1	2	3	3	1	—	—	1	—	54	
Totals	2	1	3	3	7	24	27	45	43	60	53	39	32	26	14	14	6	4	2	3	3	411	

(1) 123 samples. Mean log plate count 4·69992. Corresponding actual count 50,110.

(2) 288 samples. Mean log plate count 4·78833. Corresponding actual count 61,420.

counts exceeding 1,000,000 per g., only three occurred during the winter period. The incidence of high plate counts was sufficient to increase slightly the mean counts for May and June, but during July and August both very high and very low counts were obtained. The monthly mean plate counts actually varied little; the only noticeable features were the rather higher values obtained for May and June and the low figure obtained for April, when the best results were observed. Given satisfactory management and technical control, therefore, it is undoubtedly possible to produce high-quality powder at all seasons, even from a poor milk supply.

The occurrence of coliform-positive samples* was interesting. From November to March the monthly percentages of coliform-positive samples ranged from 37·5 to 68·4%, exceeding considerably the mean of 23·2% for all 12 months. In contrast, coliform-positive samples ranged from nil to 22·9% for the months April to October, and in general the incidence of coliform-positive samples was low during the summer months.

A tendency to attain lower hot-air inlet temperatures was observed during the winter months (Table 19), due no doubt to the low temperature of the atmospheric air supply; the milk pasteurizing temperatures were not reduced during the winter months. It has been shown that low inlet-air temperatures result in increased survival of coliform organisms, but have less effect upon plate counts, which may partly explain the above findings. Furthermore, powders produced during December, January, and February, which were dried at the lowest mean air-inlet temperatures, showed the highest percentages of coliform-positive samples.

Spore-forming anaerobes also occurred most frequently in powders manufactured during December, January, and February. These organisms are probably unaffected by variations of drying temperature, or indeed any of the factory processes, a fact which would be expected in view of their extremely heat-resistant spores. It is known, however, that they occur most commonly in raw milk during the winter months.

(10) *Variations between different products*

At a single factory Nichols(3) found that the bacterial content of separated milk powders was usually higher than that of full-cream powders. In general such results might be expected, and similar findings were obtained during the early period of operation of the two plants concerned in this work. It has been mentioned that thermoduric species may be found in increased numbers in separated milk, whilst the separating process offers obvious opportunities for additional contamination.

As has already been stated, under good conditions separated milk powders generally showed lower counts than full-cream powders. General observations, in addition to the summarized data in Table 4 and the plant runs detailed in Table 2, all indicated that full-cream milks from the pasteurizer and evaporator stages normally contained fewer bacteria than similar samples of separated milk. In spite of this, full-cream powders normally contained greater numbers of bacteria.

The full-cream runs nos. 41, 42 and 48 detailed in Table 2 reveal much higher final powder counts than would be expected on the basis of similar separated milk runs. There is a possibility, therefore, that bacterial destruction during spray drying is actually less in full-cream milk than in separated milk. If this is so, it is feasible that plants operating under poor conditions would show much higher bacterial counts in separated milk powders,

* Positive reaction in 20 ml. reconstituted milk, a severe test.

but under good conditions the counts of separated milk powders could be reduced to a lower level than those of full-cream powders. The data previously quoted (Table 1) show that differences were found between the two plants, but the plant quoted by Nichols(3) revealed much more striking contrasts in that the plate counts of separated milk powders were about eight times those of full-cream powders.

Owing to the smaller number of samples available, statistical examinations of the effects of pasteurizing and drying temperatures, and of the relationship of the various factors to moisture content and solubility, were less reliable than with separated milk powders. The influence of hot-air inlet temperatures upon bacterial quality and solubility resembled the findings for separated milk powder; the tendency towards higher plate counts and slightly reduced solubility during the progress of a drying run was also apparent with full-cream milk.

The plate counts of spray-dried whey powder were normally low, and thirty samples had a mean plate count of 29,680 per g. The five whey drying runs detailed in Table 2 show that, although the raw whey contained large numbers of bacteria, the numbers present in pasteurized and evaporated whey were not unduly large. The heat treatment employed was no different for whey, and the results were possibly due to the effects of heating in an acid medium.

(11) *Packing operations*

Earlier investigators(1) have all stressed the considerable extent to which handling and packing operations may increase the bacterial count of powder. With roller powder this is understandable, since grinding and sifting operations necessitate much handling. Spray-process powder, however, requires no grinding, and practically no handling is needed between the dryer discharge and the final container.

At the two plants under investigation the powder was mechanically removed from the bases of the drying chamber and separating cone in an enclosed conveyor (*C*, *D*, *F*, Fig. 1) which delivered it to an enclosed sifter. After sifting, the powder was packed immediately.

Pairs of corresponding samples taken direct from the dryer discharge and immediately after sifting and packing gave the following series of figures:

	Powder direct from dryer	Powder after sifting and packing
No. of samples	27	27
Mean log plate count at 37° C.	4·72855	4·80630
Corresponding plate count	53,520	64,010
No. of coliform-positive samples	4	5
No. of yeast-positive samples	Nil	Nil
No. of mould-positive samples	4	6
Mean mould count per g. of positive samples	17	18

It will be seen that the packing operations only slightly increased the mean plate count, especially when allowance is made for the standard error of the test. The coliform tests also agreed quite closely in view of the small numbers of these organisms involved. Four of the five positive packed samples corresponded to the four positive dryer samples, and the same coliform species was confirmed in each corresponding pair.

Packing operations very slightly increased the mould content of powder; during one period when an enclosed conveyor was not in use noticeable mould contamination occurred. Exposure to atmospheric contamination might also be expected to increase the numbers of micrococci and other miscellaneous organisms.

SUMMARY OF MANUFACTURING PROCESSES

The plate count of milk powder was not related directly to the raw-milk count, but was affected by the numbers of thermoduric bacteria present in the raw milk, especially when this was stored for some hours before processing; separated milk frequently contained more thermoduric bacteria than whole milk.

The pasteurization and drying operations were both high temperature-short time processes. For bacteriological reasons the minimum safe pasteurization treatment was 159° F. for 20 sec., whilst from 164 to 167° F. rather lower powder counts resulted, accompanied by slightly decreased powder solubility of no practical importance. Pasteurization at 162–163° F. for 20 sec. appeared to be a satisfactory commercial process, and yielded a pasteurized milk flora consisting mainly of thermoduric cocci.

With the drying system examined, which employed a very rapid air stream, hot-air inlet temperatures below 155° C. resulted in extremely soluble powders but were unsatisfactory from the bacteriological standpoint. Increased bacterial destruction became evident at hot-air temperatures of 165° C. or over, and temperatures up to 170° C. had no significant effect upon powder solubility. The time of exposure to hot-air drying was an important factor; bacteriological results improved but solubility showed marked deterioration as the time was increased.

The final result was a combined effect of the pasteurizing and drying processes, in both of which there was a wide margin of safety for successful operation. The normal temperature variations which occur during reasonable operation had no influence upon the quality of powder.

Some non-thermoduric organisms, e.g. coliforms and micrococci, did survive the drying process, and it would probably be unwise to rely upon spray drying for complete destruction of pathogens, although considerable destruction would occur. For this particular purpose reliance should be placed entirely upon the pasteurization process, since it was found that a completely safe pasteurization treatment, equivalent to holder treatment at 145° F. for 30 min., had no practical effect upon the solubility of powder or its nutritional value.

Henry, Houston, Kon & Osborne(14) carried out an extensive investigation of the nutritional value and vitamin content of milk powders drawn from plant 2 during the course of the present work, and reported that the spray-dried powder was equal to pasteurized milk and slightly superior to roller powder and sterilized evaporated milk.

Whilst faulty processing could be a cause of poor-quality powder, bacteriological cleanliness of the plant was a much more important factor. Accumulation of milk deposits, accompanied by proliferation of bacteria, was difficult to avoid during long runs, and the plant-cleansing problems were considerable. The evaporating process proved especially difficult; bacterial numbers sometimes decreased, but usually there was a large increase, multiplied still further by contamination and multiplication in the supply tanks feeding evaporated milk to the dryer. The increase consisted largely of thermoduric cocci, but the evaporating plant also added some general contamination, particularly coliforms and micrococci, although the effects of this upon the quality of powder were variable.

The bacterial content of powders showed a variable and irregular increase during the course of a run, which became pronounced after 10 hr.; a break for plant cleaning was desirable every 7–9 hr.

The bacterial counts at the different stages of manufacture were correlated with one

another and with the final powder counts, but there was no direct relationship between the bacterial content of powders and moisture content or solubility. Given good management plus efficient plant cleansing and sterilization, high bacteriological quality was always possible, even from a poor milk supply, and no pronounced deterioration occurred during hot-weather operation. Providing suitable precautions were taken, contamination during sifting and packing operations could be reduced to negligible proportions.

These general results differ considerably from the previous findings of Delepine, quoted by Allen(1).

Bacteriological changes during storage

Previous papers(1, 2, 3) have all recorded a decrease in bacterial numbers during storage of milk powders, and it is known that the rate of decrease is accelerated by high storage temperatures.

Earlier workers have tended to concentrate on the effects of prolonged storage periods (e.g. 12 months or more) which exceed the normal duration of commercial storage.

A series of samples was therefore stored at room temperature, in order to simulate commercial conditions, and particular importance was attached to the results of storage for 3 months.

Effects of storage upon the plate count

The data obtained from plate counts of stored samples may be summarized as follows:

	Original	3 months	6 months	12–16 months
Mean plate counts (39 samples)	51,160	13,270	1430	112
Mean % of original count	—	25·9	2·8	0·2
Range of percentages of original count	—	5·7–82·1	0·03–29·75	<0·01–9·9

The distribution of these plate counts showed the following striking changes:

% original count	3 months	6 months	12–16 months
0 – 9·9	7	29	39
10·0– 19·9	11	5	Nil
20·0– 29·9	6	5	,,
30·0– 39·9	7	Nil	,,
40·0– 49·9	1	,,	,,
50·0– 59·9	2	,,	,,
60·0– 69·9	1	,,	,,
70·0– 79·9	1	,,	,,
80·0– 89·9	3	,,	,,
90·0–100·0	0	,,	,,
	39	39	39

The detailed figures showed that the decrease in the plate counts during storage was very variable and its extent could not be predicted at any particular time. Nevertheless, it was significant that after 3 months' storage, when powder is commonly marketed, the mean count was only 25·9% of the original mean. The decrease was still more pronounced after 6 months' storage, whilst after 12 months most of the powders contained only a few spore-forming organisms.

Some individual samples yielded plate counts around 30% of the original after only 4 weeks' storage, whilst others required 4–5 months for this reduction, and it was obvious that plate counts of stored powders yielded strictly limited information. Nichols(3) has also drawn attention to this difficulty in connexion with the formulation of bacteriological standards.

The milk-pasteurization temperatures, powder-drying temperatures, original moisture content and solubility of powders bore no obvious relationship to bacterial decreases during storage, but a more extensive series of samples would be desirable to establish this with certainty.

Significant changes in the powder flora were noticed during storage. Thus *Str. durans* tended to disappear fairly rapidly and was not usually found in large numbers in powders over 3 months old, whilst *Str. thermophilus* survived rather longer. The *Micrococcus* species appeared to survive more readily than the streptococci, and constituted the bulk of the flora of many samples after 3–6 months' storage. As storage lengthened, aerobic spore-forming bacilli tended to predominate, and in many samples constituted practically the entire flora after 12 months' storage. It may therefore be inferred that the changes during storage are related to the nature of the original powder flora.

Effects of storage upon coliform organisms

Coliform organisms died out fairly rapidly in stored powders. The following series of results was obtained during storage of seventy-two presumptive positive samples:

Number of positive samples

Strain	Fresh powder	1 month	3 months	6 months	9 months
Coli I	13	2	Nil	Nil	Nil
Coli II	2	1	1	1	,,
Aerogenes I	7	2	1	1	,,
Irregular III	2	1	1	1	,,
Irregular V	1	1	1	Nil	,,
Cloacae	4	4	3	2	,,
Total true coliforms	29	11	7	5	,,
False positives due to anaerobes	43	43	43	43	43
Total all samples	72	54	50	48	43

The above figures reveal two important features: First, some species (particularly *coli* I types) died out rapidly during storage, whilst others, e.g. *cloacae* types, were more resistant; it is significant, however, that of twenty-nine true coliforms only seven survived storage for 3 months. Secondly, anaerobic spores survived for lengthy periods and the proportion of false positives therefore increased with storage. In other words, a presumptive positive reaction from a stored powder is most likely to be due to a false positive and least likely to arise from the presence of a *coli* I species.

As in the case of the plate count, therefore, the coliform test has serious limitations which should be borne in mind when stored powders are examined, and it may in fact yield no information whatever concerning the original conditions of manufacture. Such considerations might well apply, for instance, to the examination of samples of unknown age purchased on the open market.

SUMMARY

1. A bacteriological study was made of the commercial operation over a period of 4½ years of two Kestner evaporating and spray-drying plants, handling a fairly good and a very poor milk supply respectively. Processes investigated comprised handling of raw milk, pasteurization, climbing film evaporation, handling of evaporated milk, and spray drying. Data were also obtained showing the influence of variations during pro-

cessing on the moisture content and solubility of powders. Separated milk, full-cream milk, and whey were handled.

2. The general bacterial flora of spray-dried powders and the influence of individual manufacturing processes, packing and storage were studied and possible control methods suggested. Data showing the extent of both hourly and seasonal variations were also obtained.

3. A method of bacteriological analysis is described which gave reasonably good agreement between different workers.

4. Examination of 671 powder samples showed a wide variation in individual plate counts from 200 to 19,500,000 per g. The flora on standard milk agar at 37° C. was of a specialized type and comprised comparatively few species. Thermoduric streptococci of the '*enterococcus*' and '*viridans*' groups predominated, *Str. durans* and *Str. thermophilus* being the commonest species; in addition, five species of micrococci, probably non-thermoduric, also occurred commonly. Aerobic spore-forming bacilli and three *Achromobacter* species were found regularly, but their numbers were not of practical importance. Spore-forming anaerobic bacilli were present in 14% of powder samples, probably in very small numbers, and were most frequent during the winter months.

5. Coliform organisms were rarely found in 1 ml. of reconstituted milk, but were isolated from 25% of powder samples when 20 ml. of reconstituted milk was examined; coliforms occurred most frequently during the winter months. False positives due to anaerobes were common, especially in stored powders, and confirmatory tests of presumptive positives were essential. Examination of 198 coliform strains from powders and 164 strains from raw milk showed that *coli* types occurred less frequently in the powders, whilst *aerogenes-cloacae* types, particularly *cloacae*, were more frequent. 48·5% of the powder strains were heat resistant, as compared with 2·2% of the raw-milk strains. It was shown that the coliform flora of powder was partly due to plant contamination by heat-resistant strains, although some non-heat-resistant strains could survive spray drying. It seemed possible that *coli* types were less resistant to drying than *aerogenes-cloacae*.

6. Yeasts were rarely found and negligible numbers of moulds were present in 15% of powder samples. Failure to prevent atmospheric contamination during packing may further increase the mould content.

7. The plate count of powders was not directly related to the raw-milk counts, but was probably affected by the numbers of thermoduric bacteria present in the raw milk and the storage conditions before processing. Plate counts at various stages during processing were correlated with one another and with the final powder count; wide variations between individual results were obtained.

8. High temperature-short time pasteurization was satisfactory, and the heat treatment required to ensure destruction of pathogens had no adverse effect upon the solubility or nutritional value of the powder produced. Given a heating time of 20 sec., followed by a 3–5 min. holding period, a temperature of 163° F. constituted a satisfactory treatment. Temperatures below 159° F. yielded unsatisfactory bacteriological results, but higher temperatures up to 167° F. could be employed without seriously decreasing powder solubility.

9. Spray drying destroyed many bacteria, but could not in itself be relied upon to destroy pathogens, since some non-thermoduric species survived. The Kestner system

employed a rapid air stream which enabled the thermal advantages of high air-inlet temperatures to be utilized. Employing an outlet-air temperature of 90° C., it was possible to employ an inlet-air temperature of 166° C. with safety, and inlet-air temperatures up to 170° C. produced no serious deterioration of powder solubility. Inlet-air temperatures below 155° C. (commonest during winter operation) resulted in increased bacterial survival, especially of coliform organisms. Prolonged exposure of powder to hot-air currents, even at 90° C. or less (as in bag-dust collectors), reduced the bacterial content and moisture content but resulted in significantly lower solubility. Marked changes in bacteriological quality and solubility occurred at approximately similar temperature limits in both pasteurizing and drying processes.

10. Bacteriological cleanliness of the plant was a vital factor. The difficulties of each individual process have been discussed, and it was shown that opportunities were presented for contamination of plant by any heat-resistant streptococci (e.g. *Str. durans* and *Str. thermophilus*) and coliform organisms which survived pasteurization. Furthermore, conditions of operation encouraged multiplication of these organisms, and in addition it was found that contamination by miscellaneous organisms, particularly coliform species, might take place between pasteurization and drying. Particular attention was devoted to the climbing-film method of evaporation.

11. Plate counts of powders and the incidence of coliform organisms showed a variable increase during the course of a day's run, which became more pronounced after 10 hr. operation; a somewhat irregular decrease in solubility was also observed. Plant-cleaning stoppages were desirable every 7–9 hr.

12. Samples taken at pre-drying stages yielded the highest plate counts when separated milk was handled, and under poor conditions of operation separated milk powders showed higher plate counts than full cream. Under good conditions separated milk powders yielded the lower plate counts, and it seemed possible that the actual bacterial destruction during spray drying was less in full-cream milk than separated milk. Plate counts of whey powder were normally low. The general flora was similar in each type of powder.

13. Given good management and close attention to plant hygiene, seasonal variations were small and satisfactory powders were produced from a poor milk supply during hot weather.

14. Packing contamination could be reduced to very small proportions.

15. A variable decline in numbers of bacteria occurred during storage, and some species died out more rapidly than others; spore-forming species survived longest. Coliform species disappeared rapidly, particularly *coli* I types. Both the plate count and coliform tests are therefore only of limited value when applied to stored powders, especially when the conditions and duration of storage are unknown.

The authors are indebted to their colleagues A. F. Searle, R. H. Warren, and W. Whale for assistance with sampling and chemical analyses, to A. E. Walters for the drawing in Fig. 1, and to the directors of Aplin and Barrett, Ltd., for permission to publish the results of this investigation.

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(*MS. received for publication* 20 *March* 1942)

637.127.6

294. THE ESTIMATION OF SOLIDS IN MILK

PART I. DETERMINATION OF SOLIDS-NOT-FAT BY VARIOUS METHODS OF HYDROMETRY

By S. M. BODEN, *Chemistry Department, The West of Scotland Agricultural College, Glasgow,* and C. H. CAMPBELL, *Milk Department, Scottish Co-operative Wholesale Society, Glasgow*

Introduction

There are many formulae in existence for the calculation of non-fatty solids from observations made on fat content and hydrometer readings, but in this paper only two are considered. The first, Richmond's formula based on specific gravity at 60° F., was the one most widely used in this country up to 1937; the second was developed from the first and was published in a British Standards Specification(8).

It is difficult to assess the value of many of the other formulae which have been proposed, owing to a lack of explicit details regarding the techniques adopted for their derivation and the physical state of the milk samples used. One factor often overlooked is the effect of the Recknagel phenomenon, which refers to the gradual increase in density which occurs in milk on standing and which is chiefly ascribed to a slow solidification of the fat; and one criterion of reliability in a formula is that it should have been derived with a full realization of the importance of this factor. There are two obvious choices: either the milks should have been treated by heat in order to destroy the Recknagel contraction altogether, or they should have been held sufficiently long to allow attainment of maximum density. The first alternative has been adopted by Bakke & Honegger(1) and by Sharp & Hart(2), and has also found application in the preparation of the British Standard referred to above. It is therefore of interest to consider the conditions used by Richmond himself, more especially since the very age of his formula has caused its origin to be overlooked.

There is ample evidence that the pioneer workers were fully aware of the significance of the Recknagel phenomenon, although there followed a long period of neglect which ended only recently. Thus Vieth(3) in 1889 observed an average rise of 0·0013 in the specific gravity of fifteen samples when kept for 16 hr. overnight. In 1890, Richmond(4) referred to Vieth's work in the following terms: '...as Vieth has recently pointed out... the density of milk is not constant until about twenty-two to twenty-four hours after milking, a gradual augmentation taking place. It is the final density which should be taken as the normal density of the sample, as it is the one that has been assumed in the calculation of milk formulae.' Again, in 1894, Richmond & Boseley(5) wrote: 'The total solids and solids not fat can be calculated... *provided the density of the milk be not taken until after an expiration of twelve hours from the time of milking.* Before this time Recknagel's phenomenon is not complete, and calculation of the total solids may lead to serious errors.' (The italics are the authors' own; the term 'density' in these extracts should, of course, be read as 'specific gravity'.) Also in 1894, Richmond(6) noted a Recknagel

contraction of 0·0015 during 18 hr.; in the same paper he developed the formula which was presented later(7) in the well-known form of

$$\text{T.S.} = 0{\cdot}25G + 1{\cdot}2F + 0{\cdot}14,$$

although it was given somewhat differently at first (vide infra). It is important to note that although he was the author or joint author of several previous formulae, this one was developed from entirely new experimental data collected from November 1892 to October 1893, and he refers to the work thus: 'The formula deduced from these determinations was $T = 0{\cdot}2625\frac{G}{D} + 1{\cdot}2F$. It is hardly necessary to state that the maximum specific gravity was used in the calculation.'

Richmond's formula, therefore, was based on milks in which the Recknagel contraction had proceeded to completion and which had been selected throughout a twelvemonth; the importance of this second point will emerge later. Standardization of the formula was carried out by the following methods: specific gravity by the Sprengel tube; fat by a stringent Adam's coil technique; total solids by evaporation on asbestos. It follows that if the formula is to yield correct results to-day, the same conditions regarding the milk and the same, or equivalent, methods of analysis must be adopted. The construction of a hydrometer to equal the Sprengel tube in accuracy within reasonable limits is always possible; the routine Gerber method will agree satisfactorily with a gravimetric determination of fat provided the averages of a fair number of samples are compared; the choice of evaporation technique is perhaps in a different category with regard to its effect, and variations due to this cause may have considerable significance. Leaving aside the question of methods for the present, however, the physical state of the milk is of outstanding importance.

When the specification for density hydrometers for use in milk was being prepared, advantage was taken of the opportunity not only to change the basis of the hydrometry from 'specific gravity' to the more suitable 'density' but also to adopt the technique of pre-heating the milk to eliminate the Recknagel contraction. The modified formula

$$T = 0{\cdot}25D + 1{\cdot}21F + 0{\cdot}66$$

was derived from Richmond's by 'a knowledge of the coefficients of cubical expansion of milks and the density of water at 60° F.' and 'experimental determinations of the density of a number of samples of milk at appropriate temperatures, all the samples being warmed to 40° C. and kept at that temperature for about five minutes immediately prior to the density determination'; and it was claimed that the new formula would give results as reliable as the original(8). Allowing the correctness of Richmond's methods, this claim could only be justified if the calculations, by which the conversion of the old formula into the new were accomplished, included a factor to compensate for the loss of the Recknagel effect. There was no apparent indication of this compensation having been made.

Some observations made in the Milk Department Laboratory of the Scottish Co-operative Wholesale Society, Ltd., when a change was contemplated from the specific gravity technique to the B.S.S. density method, indicated the existence of discrepancies between the percentages of solids calculated by the two methods. This aroused the suspicion that the condition referred to in the previous paragraph had not been fulfilled

and the joint work to be described in this paper was carried out to put the matter to a practical test.

Methods

Source of material

The samples of raw milk consisted of individual farm supplies (made up from the mixed evening and morning yields) taken from a creamery weigh-tank. Supplies from 111 individual farms were sampled at least once in the course of investigation, the farms being situated mainly in north-west Lanarkshire where the Ayrshire breed is predominant. The average size of the herds would approximate twenty-five to thirty milch cows.

Pint samples were immediately forwarded to the Scottish Co-operative Wholesale Society Laboratory, where they were each separately mixed and divided into two portions, one lot being despatched to the Chemistry Laboratory of the West of Scotland Agricultural College while the other lot was retained. Both lots were stored at 40° F. overnight in order to ensure the maximum Recknagel contraction.

The samples of pasteurized milk (holding system) were received on the day of treatment from several creameries situated mainly in central and south-west Scotland. They represented bulked milk produced locally in these areas, the Ayrshire breed again predominating. The samples when received were mixed, divided, and stored as in the case of the raw milks.

Samples of both raw and pasteurized milks were taken during twelve consecutive months.

Laboratory technique

(*a*) *Fat*

This was determined by the B.S.S. No. 696 (1936) Gerber method, National Physical Laboratory tested glassware being employed.

In laboratory I the results were read to the nearest 0·05%; in laboratory II duplicates were accepted only if they did not differ by more than 0·05%, and the mean was recorded. In both centres, most duplicates agreed exactly.

(*b*) *Hydrometry*

The samples after being held at 40° F. overnight were warmed to about 60° F. for 's.g. unheated' (Table 1, col. 1). They were then heated as required by B.S.S. no. 734 (1937) before the determination of 'Density' (Table 1, col. 3), which was done at about 68° F. Immediately after this the samples were further cooled to about 60° F. by immersion of the bottles in water and the s.g. repeated without loss of time (Table 1, col. 2).

In laboratory I the samples were cooled from 68 to 60° F. in batches of four, and the hydrometer transferred from one sample to the next after draining but without rinsing and drying. In laboratory II the samples were cooled in pairs and the hydrometer rinsed and dried after each insertion. In both centres, the time elapsing between the beginning of cooling from 68° F. until the completion of the s.g. at 60° F. did not exceed 8–10 min., so that the Recknagel contraction had not time to commence.

In both centres, the s.g. hydrometers used had been submitted to the National Physical Laboratory and the corrections supplied were made along with the temperature corrections obtained from the table in Richmond's book (*Dairy Chemistry*, 3rd ed. p. 80). The densities

were found with no. 1 N.P.L. stamped hydrometers, corrections for instrument and temperature again being made, the temperature table on p. 25 of B.S.S. no. 734 being employed. Standard thermometers were used.

In all forms of hydrometry milk was overflowed from the cylinder as recommended to remove surface tension variations.

(*c*) *Evaporation*

Laboratory I. The method used for the first fifty or sixty samples was as follows: Quantities of milk of about 4 g. were evaporated in flat-bottomed silica basins on a water-bath until apparently dry (about 1 hr.). The basins were then transferred for about 2 hr. to a steam oven operating at 98–99° C., after which they were cooled in a desiccator and weighed. The heating was repeated for 1 hr. and for further periods of 1 hr., if necessary, until the loss in weight between consecutive weighings did not exceed 2 mg.

For the later samples, aluminium milk-bottle caps as used by Golding (9) were employed, approximately 1 g. of milk being syringed into a cap of about 1·2 g. weight. After 30 min. on the water-bath the caps were wiped individually and transferred to the oven for 2 hr. followed by repetitions of 1 hr. until the loss did not exceed 0·2 mg. The two methods gave a satisfactory agreement when tested on the same samples, and the early results can therefore be included with the remainder. The change was made for reasons of greater speed and convenience.

An Oertling analytical balance was used, not aperiodic, but quick weighing was obtained by anticipating the weight to the third decimal place, thus leaving only a small rider adjustment to be made. The samples were weighed in strict rotation and the time of cooling before weighing was kept constant. Calcium chloride and silica gel were used together as drying agents in the desiccator.

Laboratory II. The early samples were evaporated in 2 in. aluminium lids weighing about 3 g., 1 g. of milk being delivered by syringe. When superficially dry, after 15–20 min., the lids were wiped individually and heated in a steam oven at 95–96° C., first for 2 hr. and thereafter for periods of 1 hr. until consecutive heatings gave losses not exceeding 0·2 mg. An Oertling aperiodic 62 F.M. balance was employed, and time of cooling and rotation of weighing were standardized.

The later samples were done similarly, but in Golding caps, and an exact technique was evolved which gave results to 0·1 mg. Silica gel alone was almost invariably used as the drying agent in the desiccator.

In view of the findings listed in Tables 2A and 2B it may be stated here that the effect of the difference in the ovens used at the two centres was significant. While it is beyond the scope and intention of the present paper to elaborate this point further, the following observations bearing on the question of humidity may be made. It was found that after evaporation on the water-bath there remained only about 3 mg. of water per cap to be removed by oven treatment. Taking into consideration the number of caps used and the cubic capacity of the ovens, this represented 1 mg. of water per 370 ml. of oven space in laboratory I and the same quantity per 520 ml. in laboratory II. A milligram of water vapour at 100° C. occupies about 1·7 ml.

Experimental observations and results

During the investigation 237 raw-milk samples and 115 pasteurized were analysed, but in laboratory II the raw-milk number was reduced to 236 through one sample becoming sour. The results of the raw and pasteurized milks have been treated separately throughout.

The type of analytical data obtained is illustrated in Table 1, which records the results obtained in laboratory II on a random batch of samples.

Table 1

	Hydrometer readings (corr. for temp. and instrument)					% solids-not-fat found by			
No. of sample	S.G. hydrometer on milk before heating 1	S.G. hydrometer on milk after heating 2	Density hydrometer (B.S.S. technique) 3	Total solids by evaporation % 4	Butter fat % (Gerber) 5	S.G. unheated milk cols. 1 and 5 6	S.G. heated milk cols. 2 and 5 7	Density cols. 3 and 5 8	Evaporation cols. 4 and 5 9
130	30·7	29·8	27·6	12·26	3·68	8·55	8·33	8·33	8·58
131	31·2	30·6	28·5	12·05	3·40	8·62	8·47	8·50	8·65
132	31·6	30·8	28·7	12·20	3·50	8·74	8·54	8·57	8·70
133	31·2	30·5	28·4	12·07	3·40	8·62	8·45	8·47	8·67

It will be noted that on the average, the results in cols. 1 and 2 differ by 0·75, thus showing the influence of the Recknagel effect. In calculating the results in cols. 6 and 7 of Table 1, Richmond's formula was used, while for col. 8 the formula modified for the density method was used.

Tables 2A and 2B show the mean values obtained in each laboratory for raw and pasteurized milk respectively.

Table 2A. *Mean values for butter fat and solids-not-fat in raw milk*

		Solids-not-fat by			
	Butter fat %	S.G. unheated milk 1	S.G. heated milk 2	Density 3	Evaporation 4
Laboratory I (237 samples*)	3·664	8·781	8·579	8·566	8·731
Laboratory II (236 samples*)	3·657	8·765	8·569	8·588	8·783
Difference I – II	+0·007	+0·016	+0·010	−0·022	−0·052

* The averages from 236 and 237 samples are not, of course, strictly comparable; but in the present instance the error introduced into the S.N.F. was too small to affect the third place of decimals, while that of the butter fat was equivalent to only one unit in the third place. Both errors have therefore been neglected, and no corrections made.

Table 2B. *Mean values for butter fat and* S.N.F. *in pasteurized milk*

		Solids-not-fat by			
	Butter fat %	S.G. unheated milk 1	S.G. heated milk 2	Density 3	Evaporation 4
Laboratory I (115 samples)	3·729	8·779	8·566	8·561	8·738
Laboratory II (115 samples)	3·703	8·765	8·553	8·574	8·789
Difference I – II	+0·026	+0·014	+0·013	−0·013	−0·051

The following points arise from Tables 2A and 2B bearing on the ability of two observers to duplicate results, in spite of slight differences in analytical technique, when a fair number of samples are averaged.

First, in the fat estimations, excellent agreement is obtained for the raw milk, while the difference for the pasteurized (although greater than might be expected for the average of 115 samples) is only about half of the difference (0·05%) allowed for reasonable duplicates when done by the Gerber method.

Secondly, the agreement between the results by hydrometry for any one method is very close but, as will be seen from the sign of the differences, one laboratory obtained a higher mean result for the specific gravity methods while the other obtained a higher mean for the density method. This feature may be due to consistent differences in the technique of reading the hydrometers, or else to slight differences in the accuracy of the instruments, because an error of only 0·1 hydrometer degree would cause a difference greater than those found. It may be pointed out that the corrections supplied with tested hydrometers are to the nearest 0·1°.

The most noteworthy feature is the discrepancy in the evaporation results, because if the total solids are obtained by adding the butter fat to col. 4 it will be found that laboratory I was 0·045% lower than laboratory II for the raw milk and 0·025% lower for the pasteurized. Even after making allowance for the fact that errors in fat estimation are five times more important when obtaining the S.N.F. by evaporation than they are when calculating from the formulae, it would seem that the evaporation method does

Table 3. *Comparison of* S.N.F. *results by* S.G. *on unheated milks and by evaporation arranged in ascending order.* (*Abstracted from Tables* 2A *and* 2B)

Method employed	Raw milks	Pasteurized milks
Evaporation, laboratory I	8·731	8·738
S.G., laboratory II	8·765	8·765
S.G., laboratory I	8·781	8·779
Evaporation, laboratory II	8·783	8·789

not lend itself to duplication by different workers so well as does hydrometry. This fact was realized early in the investigation, and the factors causing variation in the estimation of solids by evaporation have been made the subject of further study by one of us (S. M. B.).

Turning next to consider the S.N.F. results as found by the four methods in either laboratory, it is obvious that there is a clear-cut grouping. There is general agreement between cols. 1 and 4 on the one hand and between cols. 2 and 3 on the other; but no agreement between 1 and 2 or 3, or between 2 or 3 and 4. In other words, results by our evaporation techniques agree reasonably well with those by Richmond's formula when the latter is applied to milks in which the Recknagel contraction has attained a maximum, but the agreement is lost when the Richmond or B.S.S. density formula is applied to milks which have been heated to destroy the Recknagel effect. This appears to agree with the statement made above that Richmond evolved his formula by comparing in milks at their *maximum* S.G. the results of pyknometry with results obtained by evaporation. The influence of different evaporation apparatus and techniques in different laboratories, while producing consistent results within any one laboratory, is well shown by the fact that the hydrometry results of both laboratories fall within the evaporation results of each laboratory (Table 3). Water ovens were deliberately used, since these imitated as nearly as possible the conditions likely to be prevailing at the time of Richmond's original work on the formula, but the ovens differed sufficiently in type to cause significant differences in the total solids and hence in the solids-not-fat.

STATISTICAL ANALYSIS

An endeavour was made to assess statistically the results obtained for each type of milk. To do this, the whole series of S.N.F. results as exemplified in Table 1 was employed; the results by one of the methods were taken as 'standard' results, and from these the deviations of the results by the other methods were found. These deviations then formed the variables of three series and the mean, standard deviation, standard error, etc., were calculated for each.

The choice of the method (density) which was to be the standard of reference was governed by the consideration, mentioned above, that the evaporation method may itself require standardization and also by the fact that the density method is the accepted method of hydrometry at the present time. In the preparations of Tables 4A and 4B all of the results obtained in each laboratory were employed in the calculations; there was neither subsampling nor grouping.

Statistical data for the S.N.F. *of milk samples*

Table 4A. *Raw milk*

	Laboratory I, 237 samples			Laboratory II, 236 samples		
	S.G. unheated	S.G. heated	Evaporation	S.G. unheated	S.G. heated	Evaporation
Mean difference* from density results	+0·216	+0·0127	+0·165	+0·177	−0·0198	+0·194
S.D. of mean difference	0·0455	0·0381	0·0828	0·0392	0·0237	0·0656
S.E. of mean difference	0·00296	0·00249	0·00538	0·00255	0·00154	0·00427

Table 4B. *Pasteurized milk*

	Laboratory I, 115 samples			Laboratory II, 115 samples		
	S.G. unheated	S.G. heated	Evaporation	S.G. unheated	S.G. heated	Evaporation
Mean difference* from density results	+0·218	+0·00487	+0·177	+0·191	−0·0217	+0·215
S.D. of mean difference	0·0551	0·0370	0·0937	0·0470	0·0245	0·0643
S.E. of mean difference	0·00514	0·00345	0·00868	0·00438	0·00228	0·00599

*. The mean differences recorded here may differ by one unit in the third place of decimals from those derivable by inspection of Tables 2A and 2B, owing to the approximations involved in stating the results of the latter tables to three places of decimals.

The significance between the results found by any one of the three other methods and those found by the density method, was obtained by calculating 't'. One other comparison appeared to be of interest, namely, the significance of the difference between the 'S.G. unheated' and 'Evaporation' methods. This was also calculated from Tables 4A and 4B by the 't' test. Tables 5A and 5B show these values of 't' and the conclusions drawn regarding significance.

The values of 't' obtained in Tables 5A and 5B show what was already reasonably certain from mere inspection of Tables 2A and 2B, namely, that there are very highly significant differences between the results obtained by 'S.G. unheated' or by 'Evaporation' and those obtained by 'Density' for both types of milk. It now appears that the results by 'S.G. unheated' and by 'Evaporation' also differ significantly, thus bearing out the remarks made above on the subject of evaporation methods. Finally, and least expected, in three cases out of four significant differences are found even between 'S.G. heated'

and 'Density'. As already suggested, this may arise from consistent small differences in the method of reading the dissimilar S.G. and density hydrometers, or, if the reading be correct, from errors inherent to the instruments and small enough to have escaped correction.

Table 5A. *Raw milk*

Comparisons		'*t*'	Conclusions
'S.G. unheated' and 'Density':	Lab. I	73·0	Very highly significant
	Lab. II	69·4	Very highly significant
'S.G. heated' and 'Density':	Lab. I	5·1	Significant
	Lab. II	12·9	Highly significant
'Evaporation' and 'Density':	Lab. I	30·7	Very highly significant
	Lab. II	45·4	Very highly significant
'S.G. unheated' and 'Evaporation':	Lab. I	8·3	Highly significant
	Lab. II	3·4	Significant

Table 5B. *Pasteurized milk*

Comparisons		'*t*'	Conclusions
'S.G. unheated' and 'Density':	Lab. I	42·4	Very highly significant
	Lab. II	43·6	Very highly significant
'S.G. heated' and 'Density':	Lab. I	1·4	Not significant
	Lab. II	9·5	Highly significant
'Evaporation' and 'Density':	Lab. I	20·4	Very highly significant
	Lab. II	35·9	Very highly significant
'S.G. unheated' and 'Evaporation':	Lab. I	4·1	Significant
	Lab. II	3·2	Significant

Implications towards the legal presumptive standards

In no case did either laboratory find a sample, raw or pasteurized, with a fat content below 3%. On the other hand, choice of method influenced greatly the incidence of samples showing less than 8·5% non-fatty solids. This is evident from Tables 6A and 6B.

Number (and percentage) of samples showing less than 8·5% S.N.F.

Table 6A. *Raw milk* (236 *samples*)

	'S.G. unheated' 1		'S.G. heated' 2		'Density' 3		'Evaporation' 4		No. of instances common to cols. 1 and 4	No. of instances common to cols. 2 and 3
	No.	(%)	No.	(%)	No.	(%)	No.	(%)		
Laboratory I	20	(8·5)	68	(28·8)	76	(32·2)	29	(12·3)	18	67
Laboratory II	22	(9·3)	71	(30·1)	61	(25·8)	22	(9·3)	20	61
Common to both I and II	20	(8·5)	60	(25·4)	59	(25·0)	20	(8·5)	18	55

Table 6B. *Pasteurized milk* (115 *samples*)

	'S.G. unheated' 1		'S.G. heated' 2		'Density' 3		'Evaporation' 4		No. of instances common to cols. 1 and 4	No. of instances common to cols. 2 and 3
	No.	(%)	No.	(%)	No.	(%)	No.	(%)		
Laboratory I	1	(0·9)	26	(22·6)	28	(24·3)	1	(0·9)	1	21
Laboratory II	1	(0·9)	32	(27·8)	22	(19·1)	0	(0·0)	0	22
Common to both I and II	0	(0·0)	20	(17·4)	18	(15·7)	0	(0·0)	0	17

A point arising from Tables 6A and 6B is the presence of a considerable number of 'borderline' samples. Thus in laboratory I, which usually obtained slightly higher S.N.F.S by 'S.G. heated' than by 'Density' the former method passes nine raw samples (76–67)

which are rejected by the latter; conversely, in laboratory II, where the methods worked mainly in the opposite direction (see Tables 2A and 2B), ten raw samples (71–61) which pass 'Density' fail to pass 'S.G. heated'. Similar results are seen in the pasteurized samples.

The effect of calculation from the B.S.S. formula is to return 25–30% of the raw samples, and 20–25% of the pasteurized, as being under 8·5% S.N.F.; whereas only about 10% of the raw and 1% of the pasteurized would be so returned by the 'S.G. unheated milk', or by 'Evaporation'. This is of considerable practical importance, since a laboratory which employed the B.S.S. formula for the preliminary classification of milks prior to evaporation and/or cryoscopy would be led to examine an excessively large proportion of the samples. Admittedly, so far as cryoscopy is concerned, this extra labour would detect adulterated samples which yet had over 8·5% S.N.F.; but, as the law stands at present, such findings could not be utilized. It may be noted here that the number of adulterated samples in the milks used in the present work is unknown as freezing-points were not determined.

The question also arises as to whether the gradual replacement of the old hydrometer and formula by the new, which may be taking place throughout routine laboratories, may not be capable of giving rise to impressions that milk has been deteriorating in quality (S.N.F. content) during the last two or three years. In this connexion it will be of interest to examine Table 7 which has been constructed from very large numbers of samples encountered in commercial routine.

The butter-fat column of Table 7 represents the mean value found for a total of approximately 37,000 individual composite samples in each year. The solids-not-fat column is the mean of approximately 2300 bulk samples per year.

Table 7

Year	Butter fat	S.N.F. (old formula)
1938	3·70	8·85
1939	3·68	8·78
1940	3·69	8·76
1940–1 (coincident with present work)	3·66	8·78

Summarizing our present results for purposes of comparison we have:

Table 8

	Butter fat	S.N.F.	
		Old formula	New formula
Average for raw (both centres)	3·66	8·77	8·58
Average for pasteurized (both centres)	3·72	8·77	8·57

It would seem that the samples used by us were representative of their year, although limited in number. Further, there appears to have been no appreciable difference in the S.N.F. content of milk over the last few years, *provided the results are all based on the old formula*. Adoption of the new formula in 1940–1 would, however, have erroneously indicated a deterioration in milk quality.

Influence of season upon agreement between results by evaporation and by calculation

That divergences can exist between results found by evaporation and by calculation is, of course, already well known; and they arise from causes of which evaporation technique is only one instance. A second instance is the common experience that samples from individual cows show more divergence than do the mixed milks from herds. The samples used by Richmond in deriving his formula were not actually stated to be mixed, but in view of his work as chemist to a large urban milk depot and, more especially, from the published annual reports of his laboratory, it is most improbable that they were not mixed.

There is, however, another factor which presents a barrier to agreement in many cases and which appears to be little realized: the effect of the season of the year. This was perhaps first recognized by Richmond himself(6) and it seems that only a few others have reported it, amongst whom may be mentioned Leonard(10), Koestler & Bakke(11)

Table 9. *Seasonal variations of calculated percentage values from percentage values obtained gravimetrically, as found by previous workers*

Month of year	Fat			Total solids		S.N.F. Bartlett *et al.* (means of 3 years)	
	Richmond (1891–2)	Richmond (1892–3)	Leonard* (1898)	(Koestler & Bakke, 1921–2)†		Morning milks	Evening milks
Nov.			−0·01			+0·012	+0·114
Dec.	—	—	−0·07			+0·020	+0·171
Jan.			−0·12			+0·054	+0·185
Feb.			−0·13			+0·091	+0·189
Mar.	−0·04	−0·06	−0·09			+0·061	+0·175
Apr.			−0·03	1922	+0·45	+0·015	+0·152
May			+0·02			−0·045	+0·069
June	+0·07	+0·05	+0·07			−0·024	+0·068
July			+0·11			−0·059	−0·001
Aug.			+0·12			−0·073	+0·037
Sept.	+0·02	+0·02	+0·11	1921	+0·31	−0·029	+0·071
Oct.			+0·06			−0·056	+0·043

* Calculations made by author's own formula. † Calculations made by Fleischmann's formula.

(All other calculations were by Richmond's formula.)

and Bartlett, Golding & Wagstaff(12). The seasonal divergences are sometimes positive, sometimes negative, but obviously the sum of these should be zero over the whole year provided the original formula was based upon samples taken throughout the whole year—a condition fulfilled by Richmond as stated earlier in this paper. There is considerable agreement in the findings of the various authors, supported also by work to be presented here, so that the possibility may be considered at some future time of varying a formula to suit different times of the year. It might be advanced against such a suggestion that the risk of confusion would more than counterbalance any increase in accuracy and that at best the calculation method can only remain an approximation; in any case, the factors contributing towards seasonal variation are as yet obscure.

When assessing the evidence in Table 9, it has to be remembered that Richmond and Leonard used their formulae to calculate fat, not total solids, since the direct estimation of fat was the most troublesome of all milk estimations until the Gerber and Werner-Schmid methods had become established; the later workers, of course, calculated total solids or S.N.F. Hence, variations opposite in sign or tendency in the two groups indicate similar findings.

From Table 9 it is evident that there is the same general trend throughout; minus variations in fat (or plus variations in S.N.F.) are found in approximately the same months in the experience of the different workers, and similarly for plus variations in fat (or minus in solids). Referring to the numerical differences between the variations for morning and evening milks, Bartlett, Golding & Wagstaff(12) themselves ascribe the effect chiefly to the fact that the evening samples were 14 hr. old when the specific gravity was taken (having stood overnight) whereas the morning ones were only 4 hr. old; thus the evening samples would show a fairly complete Recknagel contraction whereas the morning ones would show comparatively little. This furnishes an interesting example of the use of an evaporation technique yielding lower results than that employed by Richmond, since on the yearly average these authors obtained much better agreement between evaporation and calculation in milks showing little contraction than in milks

Seasonal variations between S.N.F. *calculated by the* 'S.G. *unheated' method and found by 'Evaporation'*

Table 10A. *Raw milk*

Period 1940–1	Laboratory I: S.N.F. by 'S.G. unheated' (1)	'Evaporation' (2)	Difference (1)–(2)	Laboratory II: S.N.F. by 'S.G. unheated' (3)	'Evaporation' (4)	Difference (3)–(4)
Nov.–Jan.	8·818	8·767	+0·051	8·813	8·839	−0·026
Feb.–Apr.	8·710	8·626	+0·084	8·693	8·691	+0·002
May–July	8·838	8·797	+0·041	8·804	8·825	−0·021
Aug.–Oct.	8·724	8·733	−0·009	8·716	8·752	−0·036

Table 10B. *Pasteurized milk*

Period 1940–1	Laboratory I: S.N.F. by 'S.G. unheated' (5)	'Evaporation' (6)	Difference (5)–(6)	Laboratory II: S.N.F. by 'S.G. unheated' (7)	'Evaporation' (8)	Difference (7)–(8)
Nov.–Jan.	8·833	8·748	+0·085	8·816	8·828	−0·012
Feb.–Apr.	8·752	8·676	+0·076	8·710	8·696	+0·014
May–July	8·738	8·710	+0·028	8·711	8·760	−0·049
Aug.–Oct.	8·725	8·797	−0·072	8·753	8·813	−0·060

with the high degree of contraction demanded by Richmond. Since, of all the groups of workers concerned, only Richmond and Leonard have described their methods with sufficient detail further conclusions cannot be drawn.

The present investigation furnished the results recorded in Tables 10A and 10B.

Considering the 'Difference' columns in Tables 10A and 10B, it will be observed that the differences in laboratory I are mainly positive in character, while those in laboratory II are mainly negative: this is largely due to the differing nature of the drying ovens in the two centres as discussed previously. The four 'Difference' columns studied separately show a tendency to rise from the first period to the second, to fall again from the second to the third, and to fall still further from the third to the fourth (however, the first period for laboratory I, Table 10B, appears to show a slightly anomalous difference compared with the other three first periods). These results are similar in nature to the findings listed in Table 9, and the evidence as a whole is strongly suggestive of the existence of predictable seasonal variations; but the factors responsible for these await elucidation.

Discussion

The work described in this paper has drawn attention to several points which are now to be summarized and discussed.

First, the specific gravity lactometer used according to Richmond's requirements (i.e. the milks to be held until maximum specific gravity has been attained) gives results for solids-not-fat about 0·2% higher than does the density hydrometer used as specified in B.S.S. no. 734 (1937) (i.e. the milks to be previously warmed to 40° C. to eliminate the Recknagel effect) when the yearly averages are compared. This is shown in Table 11.

The difference, of course, is independent of butter-fat and evaporation results and depends only upon the accuracy of the hydrometers, personal factors in the use of the hydrometers, and seasonal variations in the Recknagel contraction; in our experience, the average contraction throughout the year was about 0·8 of a hydrometer degree.

Second, it is evident that agreement between 'calculated' and 'found' S.N.F. is highly affected by the choice of the evaporation method. It seems likely that Richmond's evaporation technique would have an effect roughly intermediate to those registered by our methods (see Table 3). Many of the samples were examined by one of us (S. M. B.) using two additional ovens working at higher temperatures and 'constant' results were

Table 11

(Based on Tables 2A and 2B)

	Raw milk		Pasteurized milk	
	S.N.F. by Richmond's method (S.G. unheated)	S.N.F. by B.S.S. (Density) method	S.N.F. by Richmond's method (S.G. unheated)	S.N.F. by B.S.S. (Density) method
Laboratory I	8·781%	8·566%	8·779%	8·561%
Laboratory II	8·765	8·588	8·765	8·574
Mean results	8·773	8·577	8·772	8·568
Difference between mean results	0·196%		0·204%	

thereby also obtained, but at lower levels; and attention has already been drawn to the effect of similar severe drying (Table 9). It may well happen, therefore, that in the experience of some workers the new formula will give as good agreement with evaporation as will the old, but two considerations must be borne in mind in this connexion: (i) the comparison must be made throughout the whole year (Tables 9, 10A and 10B); (ii) the legal presumptive standard of 8·5% was fixed in the year 1901 when a milder drying technique may have been in general use. If this last supposition is *correct* then it is an argument for the retention of the old formula or, alternatively, for a moderation of present-day drying methods or, again, for a more lenient interpretation of the 8·5% standard under present-day conditions. If it is *wrong*, and if Richmond's technique was already too mild in 1901, then his formula should have been rejected from that same date. It is questionable if the 'error' should be compensated (whether by accident or design) through the elimination of the Recknagel contraction in 1937: rather should a new formula have been derived based upon the new hydrometry and a generally accepted evaporation technique. Such a technique does not exist at present and its creation is a necessary preliminary to a permanent revision of the formula.

Third, the agreement between calculated and found S.N.F. will not be constant throughout the year since the formula is the resultant of seasonal effects which are positive at

one time and negative at another. Varying the formula to obtain a closer fit is perhaps not desirable until the reasons for the effects are understood.

Summary

1. Estimations of solids-not-fat by evaporation and by hydrometry were made in two laboratories on samples of the same raw and pasteurized milks.

2. General agreement was found between the results by stated evaporation techniques and those by Richmond's formula (S.N.F. $= 0{\cdot}25G + 0{\cdot}2F + 0{\cdot}14$) when the latter was applied to milks which had been stored cold (i.e. Recknagel contraction complete as required by Richmond).

3. General agreement at a lower level of S.N.F. (0·2% approx.) was found between Richmond's formula and the density formula (S.N.F. $= 0{\cdot}25D + 0{\cdot}21F + 0{\cdot}66$) when both were applied to milks heated as required by B.S.S. no. 734 (1937) (i.e. Recknagel effect absent).

4. Adoption of the B.S.S. technique and formula placed a very much greater proportion of samples below the presumptive legal standard of 8·5% S.N.F.

5. The need is indicated for the standardization of evaporation technique before attempting a permanent revision of the density formula.

6. Seasonal variations between 'calculated' and 'found' results have been observed.

We desire to record our thanks to Prof. D. N. McArthur of the West of Scotland Agricultural College, to Mr A. McBride of the Scottish Co-operative Wholesale Society, Ltd., and to Dr N. C. Wright of the Hannah Dairy Research Institute for their helpful interest in the investigation; also to Miss Edith M. Millican of the Scottish Co-operative Wholesale Society for technical assistance in the early part of the work.

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(*MS. received for publication* 20 *October* 1941)

637.333:576.809.6

295. THE PROBLEM OF BACTERIOPHAGE IN CHEESE MAKING

PART I. OBSERVATIONS AND INVESTIGATIONS ON SLOW ACID PRODUCTION

By E. B. ANDERSON and L. J. MEANWELL,
United Dairies Research Laboratories

I. Introduction

That bacteriophage is the cause of much loss to the cheese-making industry of New Zealand has been shown by Whitehead (1). In New Zealand in recent years, cheese has been made almost entirely from flash-pasteurized milk with the aid of starters prepared from single-strain cultures of lactic acid streptococci (*Str. cremoris*). The use of such starters has been accompanied by the occasional failure of the bulk starter to clot, or, if clotted, by failure to produce the required amount of acid in the cheese vat—the latter condition being described by Whitehead as 'pack up'. Whitehead has traced the cause of sudden failure to air-borne infection of the bulk starter by specific bacteriophage (2), the most probable source of infection being the spray from whey separators. When adequate protection from phage contamination was provided slowness in cheese making disappeared.

For some years creameries in England have been subject to periods of 'slowness' in cheese making, the trouble usually being most marked during May, and occasionally to attacks of complete 'pack up'. In order to overcome this fault many cheese makers adopt the policy of adding large quantities (up to 4%) of starter to the milk. Frequently this overcomes the trouble, but it is difficult to make a cheese of good character and long keeping quality by the use of such methods. An entire change of starter usually effects a temporary and sometimes a complete cure of 'slowness' for the remainder of the season.

The investigations to be described in this paper were made mainly at creamery A of United Dairies, Ltd., where up to 25,000 gal. of milk are manufactured daily into Cheshire or Cheddar cheese (chiefly Cheshire). Except for a short period in May 1940, single-strain cultures were not used—the custom being to employ a combination of equal parts of two or three mixed commercial starter cultures, originally obtained from various sources.

II. Early indications of bacteriophage infection

Outbreaks of 'slowness' in all vats have been recorded at intervals over a period of years, particularly after the introduction of flash pasteurization of milk supplies for cheese making, but the first occasion on which 'slowness' was definitely traced to the action of bacteriophage was in April 1938. At this time, in an attempt to overcome an outbreak of May 'slowness' at one creamery, a New Zealand single-strain starter culture (Whitehead R.W.) was introduced. After working satisfactorily for several days the starter milk failed to coagulate after overnight incubation, and this starter was therefore temporarily discontinued. A supply of the culture that had failed to coagulate was examined against

a freshly revived laboratory culture of 'R.W.', and was found to be infected with bacteriophage as judged by acid production and confirmed by microscopical demonstration of lysis.

In these early tests it was found that the R.W. bacteriophage was specific to culture R.W., but that it exerted considerable influence on mixtures of starter cultures which included a percentage of this culture. It is of interest to record that a considerable lowering of acid-producing properties of the laboratory culture of 'R.W.' was noticeable immediately following the receipt at the laboratory of the phaging culture from the infected cheese factory. The figures in Table 1 show this clearly. Acid production was measured in these tests by the amount produced by a 1% inoculum of an 18 hr. culture at 22° C. of the test starter in sterilized milk after incubation for 6 hr. at 30° C.

The figures in Table 1 suggest a possible danger in introducing bacteriophage-infected material into a laboratory which is responsible for carrying cultures.

Experiments with the R.W. phaging material confirmed Whitehead's findings(3) that bacteriophage-resistant strains can be developed (in this instance by cultivation at 30° C. for 18 hr.), but the resistant strains themselves were found to be susceptible to secondary phages on reintroduction into creamery use. During these early experiments with culture

Table 1. *Acid production of laboratory cultures*

Culture	Average acid produced, expressed as % lactic acid 30° C. 6 hr.	
	14 Mar.–5 Apr.	7 Apr.–5 May
12 (Commercial mixture)	0·59	0·53
17 (New Zealand R.W.)	0·55 (min. 0·42, max. 0·66)	0·40 (min. 0·27, max. 0·61)
19 (Commercial mixture)	0·54	0·56

R.W. it was found that phaging might commence within 2 hr. of subculture, but on occasion lysis was delayed for as long as 7 hr., in which event the culture was clotted, and such apparently normal but phaging cultures might be used under factory conditions, causing 'slowness' or actual 'pack up' in the cheese vat.

III. Behaviour of freshly isolated single-strain starters when used in combination with commercial mixed-starter cultures

During the winter of 1939–40, cultures of lactic streptococci (*Str. lactis*) were isolated from sour milks obtained from different parts of the country. Some of these cultures proved to be very active acid producers at both 30 and 37° C., and also to be capable of producing considerable acid in milks that did not appear to support normal growth of commercial starters. A number of these single-strain cultures were retained for study during the cheese-making season.

Early in May 1940 when Cheddar cheese was being made from fresh flash-pasteurized milk, the usual May 'slowness' was reported by the cheese makers at two neighbouring creameries, A and B. At this time both were using in the cheese vat from ½ to 1% of a combination of equal parts of three commercial mixed starters.

Immediately on receiving the report of 'slowness' in all vats a mixture of two of the recently isolated single-strain cultures (starter H 18), which had already shown excellent

results in single vats, was introduced into the starter combination in use, one-third H. 18 and one-third each of two commercial starters.

For several days the introduction of starter H. 18 was effective in overcoming May 'slowness'. However, after the mixture with H. 18 had been in use at creamery A for 10 days, the cheese on 13 May suddenly worked very slowly, and a condition resembling complete 'pack up', as described by Whitehead, was experienced. On 13 and 14 May the cheese at the neighbouring creamery B worked normally, but a 'pack up' was experienced there on 15 May. When it was realized, on 13 May, that the A cheese were 'slow', pint cultures of starter were obtained from B (where working was normal) for the inoculation of the bulk starter for use on 14 May at A. In spite of this, and in spite of the fact that the bulk starter clotted normally after overnight incubation, the cheese at A on 14 May were very slow, whilst the cheese at B (using pints, made at the same time, by the same worker and from the same milk, to inoculate the bulk starter milk) worked normally. A consideration of these facts pointed to the conclusion that either the A milk used for bulk starter manufacture, or the A cheese vat milk, contained some inhibitory factor that was absent from the B milk supplies. The fact that the B cheese

Table 2. *Creamery A cheese records*

Date	Starter used	Vats	Making time	Grinding acidity	Remarks
12. v. 40	H. 18 + 2 mixed starters	All	5 hr. 40 min.	0·65	Normal
13. v. 40	H. 18 + 1 mixed starter	All	10 hr. 40 min.	0·60	'Pack up'
14. v. 40	H. 18 + 1 mixed starter. A bulk made from A pints	Single	10 hr. 0 min.	0·69	'Pack up'
14. v. 40	H. 18 alone. A bulk made from A pints	Single	11 hr. 15 min.	0·64	'Pack up'
14. v. 40	H. 18 + 1 mixed starter. A bulk made from B pints	Remainder	8 hr. 55 min.	0·60	Very slow
15. v. 40	H. 18 alone. Bulk made at B and used at A	Single	8 hr. 10 min.	0·40	'Pack up'
15. v. 40	One mixed starter. Bulk made at B and used at A	Single	5 hr. 55 min.	0·67	Slightly slow
15. v. 40	Four mixed starters freshly revived. Bulk made and used at A	Remainder	5 hr. 0 min.	0·70	Slightly fast

'packed up' on 15 May, suggested the possibility that the inhibitory factor may have been carried from A to B by the messenger.

A summary of the cheese-making times from rennetting to grinding at A on 12–15 May is given in Table 2. The normal time for the type of Cheddar cheese made at this creamery is 5½ hr., grinding at an acidity of 0·65%. On 14 and 15 May single vats of milk were inoculated with different combinations of starter cultures. The various combinations and the results, which are self-explanatory, are indicated in the table.

From the records at A it was obvious that starter H. 18 was responsible for the 'pack-up' condition, and after 15 May its use was discontinued and an entirely fresh combination of mixed starters was introduced. The May 'slowness' again became evident almost at once, and a second single-strain starter (starter 299, recently isolated from sour milk) was gradually introduced into the combination of starters used. On the introduction of starter 299 the cheese-making process worked normally, slightly on the fast side, until 21 May, when the later vats at both A and B 'slowed up' considerably. On 22 May a further fresh set of mixed starters was brought into use at both creameries, and there was no further trouble with general 'slowness' in all vats during the rest of the season, except on occasional isolated days.

The use of single-strain starters was discontinued from 22 May.

Throughout this investigation the quantitative effect of phage-infected material on starters has been measured by its influence on the acid production of the test starters, using a 1% inoculum of the starter in sterilized milk and incubating duplicate tubes for 6 hr. at 30 and/or 37° C., the acidity being determined by titration with $N/9$ NaOH using 1 ml. of 0·5% phenolphthalein per 10 ml. as indicator. The figure obtained is hereafter referred to as 'Activity'.

An examination of the whey from the 'pack-up' vats which included starter H. 18, and the slow vats which included starter 299, was made. To a 1% inoculum in sterilized milk of the laboratory culture of each of the starters in use on 15 and 21 May was added

Table 3. *Examination of whey from 'pack up' of* 15 *May* 1940

Starter	Test temp. ° C.	Activity: 1% laboratory culture alone	1% laboratory culture + 0·01% whey	1% laboratory culture + 0·01% boiled whey
H. 18 (single strain)	30	0·34	0·22	0·37
	37	0·38	0·20	0·38
78 (mixed strain)	30	0·41	0·45	0·47
	37	0·37	0·33	0·36
H. 18 + 78 (combination)	30	0·40	0·34	0·42
	37	0·40	0·24	0·39

Table 4. *Examination of whey from 'slow' cheese of* 21 *May* 1940

Starter (1%)	Test temp. ° C.	Activity: 1% laboratory culture alone	1% laboratory culture + 0·01% whey	1% laboratory culture + 0·01% boiled whey
299*	30	0·68	0·20	0·62
299	37	0·60	0·17	—
78†	30	0·70	0·70	0·68
78	37	0·38	0·40	0·44
(78 + 299)	30	0·67	0·41	—
(78 + 299)	37	0·30	0·19	—
236†	30	0·60	0·60	0·63
236	37	0·42	0·40	0·42
(236 + 299)	30	0·64	0·39	—
(236 + 299)	37	0·29	0·19	—

* Starter 299 = a recently isolated single strain from sour milk.

† Starters 78 and 236 = commercial mixed starters.

0·01% of whey as 1 ml. of 1/1000 dilution in quarter strength Ringer per 10 ml. milk, from a slow vat, and to a second series of inoculated milks was added 0·01% of the same whey after momentary boiling; as a control a 1% inoculum without whey addition was made. The activity was determined as described above.

Tables 3 and 4 record the results of these tests.

From Tables 3 and 4 it is evident that the addition of 0·01% whey from the cheese of 15 and 21 May to cultures of the commercial mixed starters 78 and 236 had no significant influence on activity. The effect of the whey addition on starters H. 18 and 299 was very marked at both 30 and 37° C., very little acid production taking place.

When the commercial starters were combined with starters H. 18 and 299 the failure to produce acid was also evident, being most marked at 37° C.

That the loss of activity of the starter was due to some heat-labile inhibitory factor present in the whey was confirmed by the fact that when the whey was boiled before the test it had no significant influence on the acid production of the susceptible cultures.

IV. Demonstration of bacteriophage active against commercial mixed-starter cultures

After the period of 'slowness' in all vats experienced in May 1940 no serious trouble was encountered during the rest of the cheese-making season. Occasionally on an isolated day the cheese would slow up considerably, but examination of the whey by measuring the effect of the addition of 0·1% of the whey on the activity of the starters in use, as already described above, and exclusion of the starter reacting to the whey from the bulk for a period of at least 7 days, prevented any development of the trouble. The reaction to the infected whey of one or more of the mixed-starter cultures in use on the occasionally 'slow' days, suggested that the slowness was a result of bacteriophage activity. The presence of bacteriophage active against commercial mixed-starter cultures has recently

Table 5. *'Inhibition' of commercial mixed starter* (59)

Material	Activity 30° C.	Bacterial count* 5 days at 22° C. after 6 hr. at 30° C.
(*a*) 1% fresh culture 59	0·54	310 million
(*b*) 1% fresh culture 59 + 0·001% slow culture	0·33	36 million
(*c*) 1% fresh culture 59 + 0·001% slow culture boiled	0·56	—

* All counts throughout on whey yeast extract agar.

been recorded by Nelson, Harriman & Hammer(4) and by Johns & Katznelson(5). Nelson *et al.* found that a bacteria-free filtrate from a slow butter culture proved to be inhibitory to later generations of the same culture, filtrates from bulk cultures being more powerful than those from laboratory cultures. These authors found that freshly isolated strains of *Str. lactis* were resistant to all mixed-culture filtrates tested, and from this inferred that *Str. lactis* freshly isolated from sour raw milk would be unlikely to fail under working conditions. Our experience with cultures H. 18 and 299 failed to confirm this suggestion.

That commercial mixed-starter cultures are susceptible to attack by bacteriophage was definitely demonstrated at creamery A in June 1940. One of the routine laboratory tests for starter control at that time was the determination of activity. The milk on which these tests were made was drawn from that for bulk-starter preparation which had been heated to 85° C. for 30 min., and then cooled in the creamery starter room. On 10 June a mother culture (no. 59), which had previously been carried satisfactorily in autoclaved milk, showed a sudden drop in activity at both 30 and 37° C. On the following day a 1% inoculum of a fresh mother culture was made in autoclaved milk and activity tests were carried out on (*a*) the mother culture alone, (*b*) the mother culture plus one part in one hundred thousand of the previous day's 'slow' mother culture, and (*c*) the mother culture plus one part in one hundred thousand of the previous day's 'slow' test culture after momentary boiling. The results of this examination are recorded in Table 5.

Although the fall in acid production for mixed starter 59 is not so marked as with

single-strain starters H. 18 and 299 it is evident that the slow starter did contain some inhibitory factor that prevented normal acid development and growth.

Since the reactions caused by the 'inhibitory factor' appeared to be similar to those caused by bacteriophage, an attempt was made to demonstrate its presence by the production of plaques on solid media and by lysis.

The use of Whitehead's(3) technique for plaque production was not successful on this occasion, and it was not until some months later that plaques were demonstrated for mixed starters in a phaging condition. However, lysis was demonstrated both by plate count and microscopical examination; for this purpose a 1% inoculum of a single-colony culture picked from a whey yeast extract agar plate of starter 59 was grown in sterilized milk for 3 hr. at 30° C. After this period 1% of a Seitz (EK) filtrate from the material obtained from test (*b*) (Table 5) was added to one portion. Acid production and plate and microscopical counts were determined at hourly intervals up to the sixth hour of incubation at 30° C. The results are recorded in Table 6.

Table 6. *Examination of a single colony culture isolated from mixed starter* 59

	1% culture alone			1% culture + 1% filtrate (*b*)		
Age hr.	Acidity %	Plate count 5 days at 22° C.	Microscopical count	Acidity %	Plate count 5 days at 22° C.	Microscopical count
3	0·20	86 million	68 million	Filtrate added here		
4	0·25	223 ,,	160 ,,	0·25	180 million	88 million
5	0·33	400 ,,	400 ,,	0·33	30 ,,	250 ,,
6	0·50	700 ,,	620 ,,	0·35	21,000	2 ,,

Table 7. *Examination of bulk starter and whey on a 'slow' day—*1 *September* 1940

	Activity		
Starter	1% laboratory culture alone	1% laboratory culture + 0·1% bulk starter	1% laboratory culture + 0·1% 'slow' whey
78	0·66	0·48	0·50
240	0·70	0·69	0·69

The great decrease in bacterial count combined with the cessation of acid production between the fifth and sixth hour can be taken as conclusive evidence of lysis, and therefore as strong evidence of the presence of bacteriophage as a cause of loss of activity in mixed-starter cultures.

On a number of occasions during the 1940 cheese-making season when isolated 'slow' days occurred, an examination of the bulk starter and whey, using the technique described earlier, was successful in detecting the starter responsible for slowness. When this culture was discarded and replaced on the following day by a fresh culture the cheese again gave normal working times. An example of the detection of such a mixed starter is given in Table 7.

On this occasion a combination of two mixed starters was used to inoculate the cheese-vat milk. The cheese (Cheshires) took 4 hr. to reach the required grinding acidity instead of the usual $3\frac{1}{2}$ hr. In addition to loss in activity, the presence in the whey of bacteriophage for culture 78 was confirmed both by lysis and plaque formation with colonies isolated from the culture.

V. Demonstration of plaques from infected whey

Throughout these investigations into the presence of bacteriophage in 'slow' starter cultures very many attempts were made to demonstrate plaque formation by the technique described by Whitehead(3). These were unsuccessful with both single-strain cultures and with mixed starters. However, by the following modification of the serial dilution technique plaques were produced and the presence of phage was in this manner confirmed in all infected samples examined.

Five drops (0·25 ml.) of a one-tenth dilution in quarter strength Ringer of a clotted culture of an uninfected laboratory culture of the test organism were spread over the surface of each of several whey yeast extract agar plates (3% agar). With single-strain starters the starter itself was spread on the agar plates, but with mixed cultures it was necessary first to isolate single colonies and grow cultures for spreading before plaque formation could be demonstrated. The plates were left for 30 min. to allow the moisture to become absorbed by the agar. Three drops of a dilution of a Seitz filtrate from the

Table 8. *Reaction to infected whey (Seitz filtrate) of colonies picked from a commercial mixed starter* (236)

Colony no.	Activity at 30° C.	
	1% culture alone	1% culture +0·1% filtrate
1	0·25	0·25
2	0·55	0·21
3	0·56	0·21
4	0·57	0·18
5	0·55	0·16
6	0·27	0·28
7	0·31	0·33
8	0·30	0·33
9	0·39	0·15
10	0·20	0·21

suspected material, whey or bulk starter were then spread over one-half of the agar surface of each plate. The plates were incubated overnight at 30° C., and on the following morning, if bacteriophage was present, that half of the plate which had been spread with the suspected material showed the presence of circular plaques varying in number according to the dilution and original titre of the filtrate.

It was found that some colonies picked from a mixed culture were not susceptible to the action of its infected filtrate, but that in general the rapid acid-producing colonies were those which were most susceptible. An example of the reactions to an infected whey Seitz filtrate of colonies picked from a commercial mixed culture (culture 236) are recorded in Table 8.

Culture 236 was plated on whey yeast extract agar and the colonies picked off at random into litmus milk chalk and incubated at 30° C. until clotted. The litmus milk chalk cultures were subcultured daily in sterilized milk for 3 days, being incubated for 24 hr. at 22° C. After the third subculture the test for colony-filtrate reaction was made. The filtrate used on this occasion was obtained from whey from cheese for which starter 236 had been used as one of a combination of mixed starters: an examination of the original unfiltered whey had condemned starter 236 as probably infected.

VI. SPECIFICITY OF BACTERIOPHAGE ACTION

In common with Whitehead(6) it was found that the bacteriophage-starter relationship was not necessarily specific, some filtrates being active against more than one starter culture, whilst some starter cultures were affected by more than one filtrate. The inter-relationship between starters and filtrates is illustrated in Table 9. A plus sign indicates that on the addition of 0·1% whey (Seitz filtrate) to a 1% inoculum of the starter culture a definitely lower activity was shown at 30° C. than that shown by a 1% inoculum of the starter culture alone.

From Table 9 it is clear that while the three single-strain culture-filtrate relationships appear to be specific, the mixed culture-filtrate relationships are not so well defined. This is not surprising, since all the mixed-starter cultures originally contained a number of strains of lactic organisms, some of which may have had a common origin. Further, colonies isolated from the mixed-starter cultures showed the same filtrate relationships as did the starters themselves, i.e. colonies from starter 51 were susceptible to filtrates from both starter 51 and starter 78, etc.

Table 9. *Bacteriophage-starter culture relationship*

		Bacteriophage							
Starter culture		H. 18	299	35	51	59	78	236	303
Single strain cultures:	H. 18	+	−	−	−	−	−	−	−
	299	−	+	−	−	−	−	−	−
	35	−	−	+	−	−	−	−	−
Commercial mixed-starter cultures:	51	−	−	−	+	−	+	−	−
	59	−	−	−	−	+	−	+	+
	78	−	−	−	−	−	+	−	−
	236	−	−	−	−	−	−	+	+
	303	−	−	−	−	−	−	−	+

One practical point arising from this table is that it is advisable, before introducing a fresh mixed-starter culture into a creamery where slowness is general, to make sure that it is not itself susceptible to the bacteriophage already present.

VII. THE INFLUENCE OF THE BACTERIOPHAGE OF A SINGLE-STRAIN STARTER ON A COMBINATION OF SINGLE-STRAIN AND MIXED-STARTER CULTURES

In view of the almost complete 'pack up' in the cheese vat (Table 2) experienced when a combination of starters including one infected single-strain starter was used, an experiment was devised to investigate the influence of infected whey from a single strain on (*a*) a mixed-starter culture alone, (*b*) the susceptible single-strain starter culture alone, and (*c*) a combination of both the mixed and single strains. The results (Tables 3 and 4) bear a close resemblance to the phenomenon produced by nascent phage described by Evans(7).

Evans found that the inclusion of minute traces of a phaging culture of a *Streptococcus* in a mixture of non-susceptible streptococci resulted in the lysis of the non-susceptible strains as a result of the production of nascent bacteriophage by the phaging organism. In our own experiments, while the inclusion of traces of the susceptible culture failed to have any serious influence on acid production of the combination, the influence of the susceptible single-strain culture dominated the behaviour of the combination when

proportions of single-strain and mixed starters were used which might be employed under practical cheese-making conditions. The results of the experiments described (Table 10) leave no doubt that the whey filtrate was heavily infected with a bacteriophage specific for the single-strain starter 299. The experiment was repeated with similar results on three occasions.

Two starter cultures were used, a commerical mixed culture (78) which had been found to have satisfactory acid-producing properties in both Cheshire and Cheddar cheese making, and a single-strain culture (299), recently isolated from sour milk, which had been found to produce considerable acid in milks in which commercial mixed cultures failed to grow satisfactorily, and which had also been found to have exceptionally high and early acid-producing properties at 37° C. Culture 299 had been found to be susceptible to bacteriophage in practice, and a Seitz filtrate of the whey from a slow vat of cheese containing this starter was used as the original source of phage in these experiments. These two cultures were grown alone and in combination both with and without the addition of the 299 phage filtrate.

From the figures it will be seen that at 30° C. the 1% starter combination + bacteriophage produced the same amount of acid as when 0·5% of culture 78 was used alone,

Table 10. *Examination of cultures* 78 *and* 299 *with* 299 *infected filtrate*

	Activity			Plate count			Microscopical count		
		6 hr.			6 hr.			6 hr.	
Inoculation	0 hr.	30° C.	37° C.	0 hr. millions	30° C. millions	37° C.	0 hr. millions	30° C. millions	37° C.
0·5% 78	0·15	0·45	0·35	3·15	367·0	98×10^6	4·2	586·0	190×10^6
0·5% 78 + 0·1% P	0·15	0·44	0·33	—	370·0	112×10^6	—	352·0	138×10^6
0·5% 299	0·15	0·50	0·44	10·4	2240·0	980×10^6	5·8	2000·0	410×10^6
0·5% 299 + 0·1% P	0·15	0·18	0·17	—	2·27	470,000	—	5·2	800,000
1% (78 + 299)	0·15	0·57	0·53	12·8	1200·0	700×10^6	9·6	1200·0	600×10^6
1% (78 + 299) + 0·1% P	0·15	0·43	0·18	—	218·0	400,000	—	316·0	$2·2 \times 10^6$

P = infected filtrate of 299.

whilst at 37° C. the 1% mixture + phage failed to produce any appreciable acidity at all, when the two cultures were combined the lysis of the single-strain culture (299) stopped the growth of the commercial mixed culture (78). These activity results were confirmed by bacterial plate counts and microscopical examination, when almost complete lysis was demonstrated at 37° C. for both culture 299 and the combination of cultures 78 and 299. These results in part confirm the findings of Evans (7).

In spite of apparent lysis of mixed culture 78 when grown in combination with culture 299 and its bacteriophage in the above experiments, no phage for culture 78 was recovered from the mixture after incubation.

These experiments and experiences with single-strain starters demonstrate the possible danger of introducing them into commercial cheese making to any large extent, and until some means is found of preventing their sudden failure it seems wiser to rely on multiple strain starters for creamery use.

In practice it has been found that the presence of a phaging mixed-starter culture in the combination of starters used in the cheese vat results in a condition of 'slowness' rather than 'pack up'.

VIII. THE GROWTH OF BACTERIOPHAGE UNDER THE CONDITIONS OF STARTER INCUBATION

On many occasions in the preparation of bulk starter at creamery A it was found that bacteriophage infection of the starter milk had taken place during the period of cooling before inoculation was carried out. During subsequent incubation the phage titre of the bulk starter might be expected to increase. That multiplication could take place during incubation of starter cultures inoculated into infected milk was demonstrated experimentally in the following way.

A 1% inoculum of a bacteriophage-free culture of the susceptible starter was made in autoclaved milk; increasing dilutions of its appropriate bacteriophage infected filtrate were added to this, and the phage titre determined immediately by means of the activity test at 30° C. Bacteriophage infection was considered to be present in those cultures which failed to give an activity equal to that of the uninfected control. At the same

Table 11. *Increase in bacteriophage titre of starter* 236 *after* 18 *hr. incubation at* 22° C. (16 *January* 1941)

Phage added	Unincubated culture activity	Incubated culture activity
None	0·64	0·63
10^{-4}	0·46	0·23
10^{-5}	0·52	0·24
10^{-6}	0·57	0·25
10^{-7}	**0·60**	0·26
10^{-8}	0·62	0·28
10^{-9}	0·65	0·30
10^{-10}	0·65	**0·36**
10^{-11}	0·64	0·62
10^{-12}	0·65	0·63
10^{-13}	—	0·64
10^{-14}	—	0·63

Table 12. *Increase of bacteriophage titre after incubation for* 18 *hr. at* 22° C.

Starter culture	Dilution at which bacteriophage found active: Unincubated	Incubated
51	10^{-8}	10^{-11}
59	10^{-6}	10^{-9}
78	10^{-5}	10^{-8}
236	10^{-6}	10^{-10}
303	10^{-9}	10^{-11}

time a series of tubes of the culture was inoculated with dilutions of bacteriophage filtrate varying from 10^{-4} to 10^{-14}. These infected tubes, together with uninfected controls, were incubated for 18 hr. at 22° C. After incubation the activity of the starter in all tubes was tested. It was, of course, realized that in this experiment bacteriophage would develop during the 6 hr. of the activity test. The results are, however, strictly comparative.

The results of one test of mixed starter culture 236 are given in Table 11, and a summary of all tests made on mixed starter cultures in Table 12.

Although in many instances the bacteriophage titre, as indicated by the activity test, was less than 10^{-6} in the original samples, after overnight incubation at 22° C. the titre had appreciably increased, that is to say, an infection too small to be detected in the freshly infected culture might increase sufficiently to be a cause of 'slowness' in the cheese room. Since an original infection of 10^{-5} represents an addition of less than 1 ml.

of infected whey per 15 gal. of bulk starter milk, and infected wheys taken from 'slow' cheese vats frequently have a titre of 10^{-14}, it is seen that the possibility of bulk starter milk infection with bacteriophage definitely exists.

IX. The influence of storage on phage

Experience during the 1940–1 cheese-making seasons demonstrated that once a bacteriophage infection for a starter was established in a creamery, the susceptible starter was liable to infection on reintroduction, even after its use had been discontinued for a period of 10 days.

It was desirable to obtain some indication of the life of bacteriophage in its usual carrier, whey. The following experiment was therefore made:

A fresh preparation of a single-strain starter bacteriophage (299) was made by growing the susceptible starter in sterilized milk for 2 hr. at 30° C. 0·1% of infected 299 whey filtrate was then added and incubation continued for a further 2 hr. The incubated material was coagulated by rennet, paper filtered and the filtrate passed at once through a Seitz filter. The filtrate was then added at the rate of 1% to sterile non-infected whey filtrates in which the natural acidity had been allowed to develop to 0·1, 0·5 and 0·9%.

Table 13. *Effect of storage on a preparation of phage* 299

	Effective titre of phage					
	0·1% acid		0·5% acid		0·9% acid	
Days stored	3° C.	22° C.	3° C.	22° C.	3° C.	22° C.
1	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-8}
2	10^{-7}	10^{-7}	10^{-6}	10^{-7}	10^{-7}	10^{-7}
4	10^{-7}	10^{-7}	10^{-8}	10^{-9}	10^{-8}	10^{-8}
8	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}	10^{-7}
22	10^{-6}	10^{-6}	10^{-6}	10^{-6}	10^{-6}	0
29	10^{-8}	10^{-7}	10^{-7}	10^{-6}	10^{-6}	0

The preparations were then stored at 3 and 22° C. for a period of 29 days. At intervals the bacteriophage titre was determined by the activity test.

The results are reported in Table 13, from which it is seen that prolonged storage in a relatively acid medium at 22° C. results in the destruction of phage, but unless the medium has an acidity of more than 0·5% there appears to be very little loss of strength in 29 days at 22° C. It may therefore be anticipated that, once a phage infection has become established in a creamery, outbreaks of starter failure will be liable to occur whenever the susceptible starter is used unless adequate protection is provided.

X. Phage infection in the creamery. Its detection and control

After the experiences of 1940 when 'slowness' was traced to the presence of bacteriophage-infected bulk starters in current use, a system of routine testing for the presence of bacteriophage in bulk starter and in whey was instituted at creamery A from the start of the 1941 season. This test, known as the Phage Detection Test (P.D.T.), is based on the effect of bacteriophage in reducing the activity of the starter as already illustrated. Details of the test are given in the Appendix.

Using this test throughout the 1941 season there was no serious outbreak of 'slowness'

at creamery A. Since all starters condemned by the P.D.T. were discontinued immediately on detection, it is difficult to produce concrete proof that the successful working of starters was due to the adoption of this system of control, but a study of the records provides indirect evidence in support of this view.

During the full season of 124 cheese-making days, 11 isolated days of slight slowness in all vats were experienced. Slowness was in no instance severe, the longest time from renneting to grinding for Cheshire cheese being 4¼ hr. instead of the usual 3½ hr. On 7 of the 11 slow days the P.D.T. detected at least one bulk starter which caused a fall in activity of the corresponding mother culture of over 20%, whilst on 3 of the remaining 4 'slow' days where bacteriophage was not detected, it is possible that the starters used were not in a naturally active state.

In addition to the 11 slightly 'slow' days, bulk starters showed a drop of over 20% in the P.D.T. on nineteen occasions without causing slowness in all vats.

In Table 14 a summary is given of the phage detection tests carried out at creamery A during 1941. The days are divided into two groups: first, those on which all vats worked normally or only an odd vat was slow, and secondly, those on which some slowness was present in all vats.

Table 14

	Normal days (or odd vats slow) Activity drop in P.D.T. 30° C.					'Slow' days Activity drop in P.D.T. 30° C.				
	0	⅓10%	⅓20%	⅓30%	>30%	0	⅓10%	⅓20%	⅓30%	>20%
Bulk starters	325	17	23	11	10	23	3	3	3	4
				5·4%					19·5%	
Days	60	13	21	9	10	3	1	0	3	4
				16·8%					63·5%	

In the upper half of the table all the bulk starters examined are classified according to the percentage reduction in activity, while in the lower the days are classified according to the worst bulk starter occurring on any particular day.

It will be seen from these figures that on 'slow' days a considerable drop in activity of the bulk starters, i.e. of over 20%, was relatively much more frequent than on normal days, and similarly that 'slow' days were relatively more frequent when this high drop occurred.

It is still not clear why normal days should occur with highly infected bulk starter and 'slow' days when bacteriophage was absent. In the former, experience suggests that the two active mixed starters present in the combination used were able to mask the low activity of the phaging mixed starter. On the three 'slow' days it had been unavoidable to use starters known to be relatively sluggish.

Apart from this, it is evident that, despite the control system employed it was not possible entirely to exclude bacteriophage infection under the conditions existing at the time.

XI. PROTECTION OF BULK STARTER FROM BACTERIOPHAGE INFECTION

Whitehead & Hunter(2) have reported that at the Bell Block Creamery in New Zealand where bacteriophage infection was very severe, the trouble has been overcome for one

season by the provision of a separate building for starter preparation. Whitehead also suggests the possibility of avoiding bacteriophage infection by the cultivation of bulk starter in airtight containers provided with a small opening, plugged with cotton-wool, through which the milk can be inoculated. Since war-time conditions render impossible the provision of separate buildings sufficiently protected from air-borne contamination, a series of tests on the manufacture of bulk starter in such a type of starter can, using heavy aerial contamination of the bacteriophage of the culture used, has been carried out with very promising results. These results are recorded in Table 15. The figures represent the activity of the mother culture of the starter used, and the same with addition of 0·1% of the bulk starter from the two types of can.

Table 15. *Phage detection test of experimental bulk starters* (*no.* 338)

Test	Activity							
	30° C.				37° C.			
Date ...	23. xii. 41	24. xii. 41	25. xii. 41	5. i. 42	23. xii. 41	24. xii. 41	25. xii. 41	5. i. 42
1% mother culture	0·70	0·61	0·59	0·44	0·42	0·38	0·49	0·39
1% mother culture + 0·1% A	0·72	0·62	0·61	0·44	0·41	0·41	0·55	0·42
1% mother culture + 0·1% B	0·55	0·41	0·31	0·43	0·30	0·30	0·19	0·36

A = bulk starter from special starter can. B = bulk starter from ordinary can.

From Table 15 it is seen that in every test the bulk starter from the ordinary starter can showed the presence of bacteriophage at both 30 and 37° C., whilst that from the special can showed a lowering of activity of 2% on one occasion only, a figure within the limits of experimental error.

XII. Discussion

All Whitehead's findings for single-strain starters have been found to apply equally well to the mixed starters in common use, although the failure of mixed starters is usually not so marked as that described by Whitehead for single strains.

Starter troubles in England may have in the past been masked by the use of raw milk which has enabled sufficient acid to be produced from the natural flora in spite of partial starter failure, but the quality of the resulting cheese has often been very uneven.

The present paper presents strong evidence that phaging of mixed-starter cultures is a very important factor in the occurrence of 'slowness' in cheese making. Whether the exclusion of bacteriophage infection from bulk starters will entirely cure general slowness is still an open question. Whitehead & Hunter (2) report freedom from slowness even when bacteriophage is demonstrated in cheese making, providing that the bulk starter is free; on the other hand, Johns & Katznelson (5) report 'pack up' as a result of the infection of cheese rakes with phaging material.

In our experience 'slowness' has been found only when the bulk starter itself has been infected, and on many occasions bacteriophage-infected whey has been found in association with uninfected bulk starter which has given a normal cheese working time. For control purposes whey infection is regarded as a warning that the particular phage is present in the creamery.

Droplets of infected whey may easily gain access to bulk starter milk, and a small

whey infection on one day may result in starter failure on the following day. In the preparation of bulk starter every precaution should therefore be taken to avoid contamination, as, for instance, by the use of a special type of starter can.

It is obvious that many interesting and important problems arising out of this investigation remain to be solved, and on these work is proceeding.

XIII. CONCLUSIONS

1. Failure of single-strain starters as a result of bacteriophage infection has been confirmed in this country.
2. The inclusion of certain single-strain phaging starter cultures in a combination of starters in the cheese vat will cause 'pack up' or very serious 'slowness' in cheese making.
3. Mixed culture cheese starters are also subject to failure as a result of bacteriophage infection.
4. Bacteriophage for mixed culture starters is not altogether specific.
5. The phaging of certain single strain starters may prevent the growth of non-susceptible starters.
6. A minute infection of bulk starter milk with bacteriophage will increase one thousandfold after overnight incubation in the presence of the susceptible starter.
7. Bacteriophage of mixed starter cultures will retain its full strength on storage.
8. A system of starter control by means of a 'Phage Detection Test' is described.
9. The protection of bulk starter from bacteriophage infection has been carried out successfully.

Our thanks are due to the Zone, Factory and Production Managers for their valuable help and co-operation throughout the investigation, and to the Directors of United Dairies for permission to publish.

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APPENDIX

Technique of phage detection test (P.D.T.)

Apparatus

Water bath, thermostatically controlled at 30° C.
Reductase tubes, sterile.
1 ml. pipettes, sterile.
Dippers, sterile.
Bottles sample, sterile.

Materials

100 ml. quantities of milk autoclaved at 15 lb. for 20 min.

9 ml. tubes of Ringer solution, quarter strength.

Sampling

Examine a single pint and single bulk can for each starter and whey from one vat. Sample the bulk starter immediately the lid is removed and before plunging using a sterile dipper and a sterile bottle. Samples should be stored in the refrigerator until the series is complete, and then put on for test.

Method

The test must *not* be carried out in the starter preparation room. The test is applied to each starter in use at the following stages:

A. Mother culture.

B. Pint culture for inoculation.

C. Bulk starter.

D. Whey at cutting corresponding to C.

To 100 ml. of autoclaved milk add 1 ml. of A, mix carefully and distribute in 10 ml. quantities in 8 sterile reductase tubes per starter to be tested.

To each of 2 add 1 ml. sterile Ringer solution = Control (*a*).

To each of 2 add 1 ml. of 1/100 dilution of B = (*b*).

To each of 2 add 1 ml. of 1/100 dilution of C = (*c*).

To each of 2 add 1 ml. of 1/100 dilution of D = (*d*).

Place the tubes in the water-bath simultaneously. At the end of 6 hr. remove and determine the acidity, transferring the contents to a 150 ml. flask and rinsing out with 10 ml. distilled water.

Application

If the mean acidity of (*b*), (*c*) or (*d*) is more than 10% less than that of (*a*), phage may be presumed to be present in the culture under test.

Note. A few phages are more active at 37° C. than at 30° C. If the test at 30° C. fails to reveal the presence of phage in one or more of the starters, repeat at 37° C. All apparatus used for this test must be handled separately from other bacteriological apparatus, pipettes and test tubes must be soaked overnight in a solution of sodium hypochlorite (1 in 500) before washing. Milk which has been used in the test must be poured into a container kept for this purpose only and autoclaved before disposal.

(*MS. received for publication* 3 *March* 1942)

637.333:541.18

296. THE EFFECT OF VARYING PITCHING POINT AND RATE OF SCALD ON THE PHYSICAL PROPERTIES AND GENERAL QUALITY OF CHEESE MADE FROM MILK OF VARYING ACIDITY AND FROM PASTEURIZED MILK

By G. W. SCOTT BLAIR, F. M. V. COPPEN and D. V. DEARDEN,
National Institute for Research in Dairying, University of Reading

(With 1 Figure)

Introduction

In an earlier paper(1) an account was given of a preliminary experiment on the effect of controlled differences in consistency of curd at pitching on the physical and chemical properties of the ripened cheese as well as on the properties of the cheese as judged by experts and others. The effect of varying the rate of scald was also found to be marked. In the light of these preliminary findings, a further experiment was planned to study more fully these effects.

For the present experiment five vats, each with 50 gal. of milk, were made into cheese on two successive days. The milk used was taken from the same source of supply each day and was commercially pasteurized. The milk was divided so that the composition and quantity in each vat were similar.

The vats of milk, for cheese 1–5, were each inoculated with 0·25% starter and a ripening period of half an hour allowed. For cheese i–v starter was added at the rate of 2·5% with a ripening period of an hour. The differences in quantity of starter and length of ripening period were made to give a low acidity at renneting on the first day and a comparatively high one the following day. These will be referred to as 'sweet' and 'acid', although the working of the latter would not be considered acid from a cheese-making standpoint. The rate at which the temperature was raised during the scald was normal for cheese 5 and v, rapid for cheese 1, 2, i, ii and slow for cheese 3, 4, iii, iv. The curds of cheese 5 and v were pitched at a normal consistency for Cheddar, 1, 3, i, iii comparatively soft and 2, 4, ii, iv on the firm side. The pitching points were determined by the Scott Blair-Coppen method. Apart from these variations, the time intervals and general management of the curd were kept as nearly as possible the same for each vat of milk. The curd from each vat at hooping time was made into two cheese numbered I and II.

The essential differences in manufacture are shown in Table 1.

The cheese were all ripened under the same atmospheric conditions. One cheese of each pair (I) was reserved for chemical analysis and physical tests during the ripening period. It was planned to do six of these tests so that for each, one-sixth of the cheese was required. After the first and second samples had been taken, cheese wax was used to replace the portion of cheese removed, so that the cheese was given support as well as protection from loss of moisture. After taking the remaining samples sufficient protection was given by applying wax to the cut surface only.

The second cheese (II), kept for the final tests and judging, had a half reserved for examination by the cheese judges, a piece across kept for physical tests and chemical analyses, and the remaining portion cut into cubes (about $1\frac{1}{4}$ in.) to supply three pieces from different parts of the cheese for each subjective test.

For the subsidiary experiment, 12 gal. of raw milk were divided into four equal portions. (A) was kept raw and the other three pasteurized to (B) 150° F., (C) 165° F., (D) 180° F. In the absence of a small enough standard pasteurizer, pasteurizing was done by standing the churn of milk in water heated by steam injection. During heating the milk was stirred and, on attaining the required temperature, cooled to 84° F. Half a pint of starter was added to each lot of milk, and a ripening period of an hour allowed before renneting. Size of cut and rate of scald were kept as nearly alike as possible.

Curd D did not firm up and could not be used for the experiment.

Table 1

Cheese no.	Titratable acidity at renneting	Time from cut to max. temp. hr. min.	Time from max. temp. to pitch hr. min.	Titratable acidity at pitch	Pitching consistency	Titratable acidity after salting
1	0·145	31	30	0·09	21·4	—
2	0·145	32	1 9	0·09	19·5	—
3	0·145	1 7	10	0·10	21·8	—
4	0·145	1 3	41	0·095	19·1	—
5	0·14	43	29	0·095	20·8	—
i	0·17	27	20	0·14	21·4	0·60
ii	0·17+	27	1 14	0·185	19·2	0·67
iii	0·17+	1 7	2	0·15	21·5	0·55
iv	0·17	1 5	33	0·18	18·7	0·68
v	0·17	45	30	0·16	20·2	0·53

Main experiment

Physical and chemical tests on curd and cheese (objective tests)

In the light of experience from the earlier experiment, some modifications were made in the list of physical and chemical tests. For example, chemical analyses for phosphates, calcium, chlorides, determinations of moisture content of the curd at pitching, and some of the titratable acidities were found to be of little interest. Measurements of *p*H gave unsatisfactory results and are therefore not included. The testing of the finished cheese by means of the spring skewer was also not repeated because of lack of facilities for getting an apparatus made with sufficient accuracy. It is believed that the somewhat disappointing results obtained with the home-made instrument were due to faulty bearings.

For further details of the following tests, the reader is referred to the earlier paper, the same Greek letters being used.

(δ, ϵ)* The vapour pressures and moisture contents were measured five times during the ripening process on the first set of cheese (I) and twice more on the second set (II), which had been kept for subjective judgements when ripe after the judging was completed. All subsequent tests on the ripening cheese were done six times on cheese I and twice on cheese II.

(ζ, ρ, σ) In this experiment the ball compressor was used. The data recorded were

* We are indebted to Mr B. Veinoglou for these tests.

total deformations and amount of recoverable (elastic) and non-recoverable (plastic) deformations.

(λ) The pitching number(2) and the rate of scald.

(μ) Titratable acidity after drawing off whey.

(ξ) Titratable acidity at pitching.

(ϕ)* 'Firmness' determined by means of the rheometer on cylinders cut from the cheese. All rheometer tests were done at two shear stresses, 3·28 and $6{\cdot}18 \times 10^4$ dynes/cm.^{2}

(ψ)* Amount of elastic recovery on rheometer.

(ω)* Coefficients of dissipation (rheometer).

In addition, in the new experiment, we recorded:

(a) Time of scald.

(b) dk/d (log S). The coefficient of dissipation (k) should theoretically be independent of stress(3) (S). In fact, it was found that there was a tendency for the softer cheese to show a lower dissipation coefficient at low stresses than at high stress. It is found empirically that curves plotting $k/\log S$ are linear. The values of dk/d (log S) were calculated. As the cheese firmed up on ripening, the values of this coefficient fell progressively, reaching zero and in some cases changing sign. Crumbliness was not considered in the present experiments, since many of the difficulties caused by it in the earlier work have now been overcome.

Judgements on the ripe cheese (subjective tests)

The cheese (II) which had not been cut up for analysis were examined by three types of subject, namely, expert cheese makers (e), non-experts (n), and routine analysts (r). This last category replaces the bakers' category of the earlier experiment, since psychophysical tests have shown(4, 5, 6) that routine analysts are frequently superior in judging firmness and similar properties of soft materials. All the subjects were given the following tests to do.

Three approximately $1\frac{1}{4}$ in. cubes were cut from random portions in each cheese and the fifteen cubes so obtained from each half of the experiment were presented to the subject who was asked to place them in order of firmness and subsequently in order of 'spring'. A separate set of cubes was used for each subject. Many subjects expressed difficulty in assessing 'spring' using the cubes, so the judgements for 'spring' were repeated by pressing with the thumb on the whole cheese.

In addition, the cheese makers alone were asked to examine the cheese in the usual way, scoring separately for body, texture, colour, taste and smell. A composite order for general grading score was also calculated from these data by allowing all the factors equal weight except colour which was given half the value of the others. Experts were asked to grade the cheese in each case from 10 for the best to 1 for the worst, but in practice they tended to give intermediate figures which differed little from cheese to cheese.

* For a description of the rheometer technique vide Scott Blair & Coppen(3).

Results of the tests

(1) *Interrelationship between results of the subjective judgements*

(*a*) *Cheese makers' judgements of general grading score*

Table 2 gives the orders of the cheese for each cheese maker for general grading score.

Table 2

Cheese maker	Sweet curd					Acid curd				
A	2	4	3	1	5	v	iv	iii ≒	ii	i
B	1	5 ≒*	2	4	3	i	ii ≒	iv	v	iii
C	4	1	2	3	5	ii	i	v	iv	iii
D	2	5	4	3	1	v	iii	ii	iv	i
Mean order	2	4	5	3	1	v	ii	iv	i	iii

* ≒ means 'very slightly greater than'. 'Equals' are written with horizontal bracket below.

These results show very wide discrepancies between the judgements of the four individual cheese makers on general grading score, i.e. on what may be called the 'value' of the cheese (the word 'quality' is avoided because of its special meaning). From what follows it will be seen, however, that as far as judgements of firmness and 'spring' are concerned, the cheese makers show their superiority over other categories.

(*b*) *Firmness*

(i) *Self-consistency of individual subjects.* A mean position for each cheese was calculated for each subject from the positions allotted to each of the three replicate samples. From these a general mean position was calculated for each group and for all three groups taken together. The average deviations for each subject for all the cheese and for each cheese for all subjects within each group were also calculated.

The average deviations on all cheese (sweet and acid taken together) for each subject are given in Table 3.

Table 3

Subject	A	B	C	D	E	F	G	H	J	K
Average deviation	2·31	2·05	2·08	2·59	3·07	2·80	3·33	3·19	3·35	2·77
Category			*s*			*n*			*r*	
Average for category			2·26			3·07			3·10	

It will be observed that there is no single non-cheese maker ($n+r$) who has been as self-consistent as even the least self-consistent of the cheese makers. The difference between non-experts (n) and routine testers (r) is clearly insignificant.

In the earlier experiment, this point could not be tested, since replicate samples were not given. Since this result is quite different from those previously obtained for experiments on materials other than cheese we conclude that cheese makers have acquired some specific ability to reproduce judgements for what they believe to be firmness in cheese, but that this ability is not an increased general sensitivity nor does it necessarily bear any relation to 'firmness' itself as measured by physical means. They are able, in fact, to pick out replicates from a series of cheese samples with more success than can

members of the other categories. The relationships with objective physical data are discussed in a later section.

(ii) *Self-consistency of groups.* The next question which arises is whether cheese makers taken as a group are in closer agreement one with another than are non-experts and routine analysts taken as groups.

In expressing the data, one difficulty immediately presented itself. Imagine a group of subjects giving a completely random order, then the mean positions given to the ten cheese (taking averages from the three replicates) will all fall very close to the middle of the scale. In other words each individual differentiates between the cheese to such a very small extent that it would be impossible for any group of such individuals to differ among themselves to any very great extent. In other words a group consisting of individuals having a low level of self-consistency will tend, as a group, to show a high self-consistency. To get over this difficulty, it is necessary to scale up the results for such individuals so that all individuals cover the same range. The simplest way of doing this is to use the rank orders obtained from the mean positions instead of the mean positions themselves. In this way we calculated the average deviation for each separate category and found for *e*, 1·29, for *n*, 1·69 and for *r*, 2·07.

This shows that the cheese makers are not only more self-consistent as individuals but also, in so far as what they believe to be firmness is concerned, they agree better among themselves than do members of the other two groups.

(*c*) *'Spring' (on the cubes)*

(i) *Self-consistency of individual subjects.* Following the same procedure as for firmness, we find an average deviation of, for category *e*, 2·51, for *n*, 2·50 and for *r*, 2·95. In this case, there was a fair amount of overlapping between the categories. It seems probable that the routine analysts are definitely worse than the other two groups, but cheese makers and non-experts do not differ.

(ii) *Self-consistency of groups.* Again, using the same methods as for firmness, the following average deviations are found: *e*, 1·67; *n*, 2·13; and *r*, 1·82. Here again the experts appear superior, the routine analysts being intermediate between them and the non-experts.

Two of the cheese makers (C and D) were unable to distinguish the orders for spring and firmness, and it seems doubtful whether they really grasped the essential differences between these two properties. Moreover, some subjects found such difficulty in assessing 'spring' that they grouped many of the cheese together as 'equals'. These two facts undoubtedly limit the significance of the results with respect to 'spring'.

We would emphasize again that all the conclusions in this section refer to self-consistency only and give no indication as to the capacity to measure firmness or 'spring' in agreement with the physical facts.

Results of physical and chemical tests (objective tests)

(*a*) *Variation of properties during ripening*

The only property which normally increased during ripening was firmness. In the sweet curd the dissipation coefficient tended to increase, but in the acid curd it increased only at low stress, tending to decrease at the high stress. All other properties decreased except that the variations in dk/d (log S) were somewhat irregular.

If we take as a measure of rate of ripening, the percentage change, in the above sense, between the average values for the first three and the second three tests for all these properties (neglecting dk/d (log S)), we can arrange the cheese in order of rate of ripening, though this order refers to the physical changes in ripening and not to the more usually considered chemical ones. The order so derived (C) is 3 : 2 : 5 : 1 : 4 for the sweet curd and iii : ii : iv : v : i for the acid curd.

It is interesting to note that this order is not correlated with either the pitching consistency or the rate of scald. The average percentage change for acid curd was 9·8 and for sweet curd 5·2: the acid curd ripened almost twice as fast.

The cheese numbered I were almost entirely used up in getting samples for the six tests (five in the case of vapour pressure). This meant that the unused portions might hardly be expected to be ripening in a normal way. In order to find out whether this seriously affected our tests, the other cheese (II) were tested twice after they had been used for the subjective judgements. Each of the physical properties measured was plotted against time since making, and it appeared that there was no obvious break or change in position associated with the change over from cheese I to cheese II. When the averages were worked out, however, it was found that, for tests 7 and 8 (cheese II) these differed more from those for tests 4–6 and 1–3 (cheese I) than did those from one another. We conclude that the sampling effect was significant but not serious enough to vitiate our results.

(*b*) *Comparison of rank orders derived from subjective and objective tests*

Tables were drawn up as in the earlier work in which the cheese were placed in order of magnitude for each of the properties concerned. As before, the properties are arranged in two columns in such a way as to bring the most nearly identical orders together, both within and between the columns. The experiments on sweet and acid curd were taken quite separately and are given in Tables 4 and 5 respectively. In most cases, experiments were done in duplicate on each day and the rank order was derived from the means of the results for all the experiments done on cheese I, i.e. means of five or six days. Data from the later objective experiments on cheese II are not included.

As before, where several tests seem to fall into a natural group, they are bracketed, and lines linking the two sides of the table emphasize similarities which might otherwise be overlooked. Orders are inverted where this process emphasizes similarities.

Discussion on main experiment

Contrary to the tentative impression which was recorded in the earlier paper that rate of scald might be as important as the pitching consistency, it now appears that far more properties of the cheese are governed by the latter than by the former, though there is some evidence that, as previously suggested, a more rapid rate of scald produces a springier cheese, at least as judged by subjective means.

Consistency at pitching is closely correlated with firmness, moisture content, dissipation coefficient, elastic recovery and ball compressor firmness (i.e. reciprocal of total deformation). The rheometer orders agree remarkably well whether derived from the higher or lower stress figures.

Rate of scald is correlated with vapour pressure in the case of the acid curd, but in the case of the sweet curd the higher rate of scald gives a curd of lower vapour pressure.

Table 4. *Sweet curd*

Subjects' category	Subjective tests: Test	Rank order					Objective tests: Rank order						Test
e	Spring (whole cheese)	2	4	1 : 5		3	2	4	5	1	3	ϵ	Inverse moisture content
n	Inverse spring (whole cheese)	2	4	3	1	5	2 ≒ 4		5	1 ≒ 3		λ	Inverse PITCHING NUMBER (firmness at pitch)
e	Taste and smell	2	4	3 : 5		1	2	4	5	3	1	ψ	Inverse recovery (rheometer high stress)
e	Grading score	2	4	5	3	1	2	4	5	3	1	ψ	Inverse recovery (rheometer low stress)
e	'Body'	2	5	4	3 : 1		2	4	5	3	1	ω	Inverse dissipation coefficient (low stress)
e n r	Firmness	2	3 : 4		5	1	2	4	5	3 : 1		ω	Inverse dissipation coefficient (high stress)
n	Inverse spring (cubes)	3	2	4 : 5		1	2	4 : 5		3	1	ϕ	'Firmness' (rheometer high stress)
e	Inverse spring (cubes)	2	3	5	4	1	2	4 : 3		5	1	ϕ	'Firmness' (rheometer low stress)
e	Firmness	2	3	5	4	1							
r	Spring (cubes)	4	2	3	5	1	4	2	3	5	1		Scald time
n	Firmness	4	2	3	1	5	2	5	4	3	1	b	Inverse rate of increase of dissipation with stress
r	Firmness	4	2	1	3	5	2 : 5		4	3	1	ρ	Inverse total deformation (ball compressor)
							5 : 4		2	3	1	ζ	Inverse plastic deformation (ball compressor)
							2 : 5		3	4	1	σ	Inverse elastic deformation (ball compressor)
e n r	Spring (cubes)	3	2	5	1	4	3	2	5	1	4	c	Rate of ripening
							4 : 3		5	2 : 1		a	Inverse rate of scald
e n r	Inverse spring (whole cheese)	3 : 4		1	2	5	3	5	4	1	2	δ	Vapour pressure
							3	4 : 5		1 : 2		ξ	Acidity at pitch
r	Spring (whole cheese)	2	3	1 : 5		4							

Table 5. *Acid curd*

Subjects' category	Subjective tests: Test	Subjective tests: Rank order					Objective tests: Rank order						Objective tests: Test
e n r	Spring (cubes)	ii	i	iv	iii	v	ii	i	iii : iv	v	ζ		Inverse plastic deformation (ball compressor)
r	Inverse spring (whole cheese)	ii	i : iv		iii	v	ii	iv	iii	i	v	ρ	Inverse total deformation (ball compressor)
n	Firmness	ii	i	v	iv	iii	ii	iv	iii	v : i		σ	Inverse elastic deformation (ball compressor)
							ii	iv	v	iii	i		Acidity at 'whey off'
n	Inverse spring (whole cheese)	ii	iv	v	iii	i	ii	iv	v	iii	i		Scald time
							ii	iv	v	iii	i	ξ	Acidity at pitch
e	Inverse spring (cubes)	ii	iv	i	v	iii	iv	ii	v	iii	i	ε	Inverse moisture content
e n r	Inverse spring (whole cheese)	ii	iv	i	v	iii	iv	ii	v	i	iii	λ	Inverse PITCHING NUMBER (firmness at pitch)
e	Firmness	iv	ii	i	v	iii	iv	ii	v	i	iii	ψ	Inverse recovery (rheometer high stress)
e n r	Firmness	iv	ii	i : v		iii	iv	ii	v	i	iii	ψ	Inverse recovery (rheometer low stress)
e	Inverse spring (whole cheese)	iv	ii : v		i	iii	iv	ii	v	i	iii	φ	'Firmness' (rheometer high stress)
r	Firmness	iv	v	ii	i	iii	iv : ii		v	i	iii	φ	'Firmness' (rheometer low stress)
r	Spring (cubes)	iv	v	ii	i	iii	iv : ii		v	i	iii	ω	Inverse dissipation coefficient (high stress)
e	Grading score	v	ii	iv	i	iii	iv : ii		v	i	iii	ω	Inverse dissipation coefficient (low stress)
							v	iv	ii	i	iii	b	Inverse rate of increase of dissipation with stress
e	Inverse 'body'	iv	iii	v : i : ii			iv : iii		v	i : ii		a	Inverse rate of scald
							iv	iii	ii	i	v	δ	Inverse vapour pressure
n	Spring (cubes)	v	iii	ii	iv	i	iii	ii	iv	v	i	c	Rate of ripening

The rate of ripening (physical) is not very closely related to any other property but, with acid curd, there appears to be some inverse relationship with vapour pressure. This is not apparent in the sweet curd. It is rather remarkable that rate of ripening does not appear to depend on firmness or moisture. As already noted, the rate of ripening was much higher for acid than for sweet curd.

The acid curd showed a marked relation between acidities at pitch and 'whey off' and time of scald but this was not apparent in the case of the sweet curd. The differences in acidity between the sweet curds were extremely small and the lack of correlation is not surprising.

Although no attempt was made to arrange the properties in the same order in the two experiments (sweet and acid), it will be seen that there is a marked similarity in the orders on the right-hand side of Tables 4 and 5. For self-consistency, the superiority of the experts, individually and as a group, has been established. As far as correlation with physical tests is concerned, with the sweet curd, the experts again appear to correlate somewhat more closely than do other categories with the rheometer figures, though there is much confusion between firmness and 'spring'. It is interesting to note that taste and smell correlate well with rheometer data, bearing out the suggestion that these properties are largely dependent on rheological conditions. In the case of the acid curd, there was too little differentiation between the cheese in taste and smell for the corfelation to be observed.

These conclusions appear to conflict somewhat with those given in the earlier paper, and we must examine how far this apparent difference is significant. It must be remembered that in the present experiments the cheese experts did best in judging the sweet curd, which was quite unlike the material used in the earlier experiment. In the case of acid curd, the only real difference appears to be that in the present experiments the non-expert group agreed less well with the objective tests than they did in the earlier experiment. The same experts took part in the two experiments, and it might be suggested that the previous difficulty which they experienced in isolating firmness from other factors may have been partly overcome by practice. Of the non-experts, only one of the present panel served in the earlier experiments and also the group of non-experts in the earlier experiments contained two routine testers, the distinction not having been made on that occasion. Moreover, in the present experiment, especially with the sweet curd on which the experts did so much better than in the earlier experiment, the properties of importance in cheese grading, other than firmness, correlated highly with firmness itself, so that the confusion was much less likely to occur.

One purpose of the present experiment is to establish what physical measurements give the most useful measure of properties important in cheese making and grading.

We conclude in general that the rheometer gives the best measure of the more important physical properties of cheese though we do not yet fully understand the interpretation of the data, particularly the significance of dissipation coefficients. It is hard to say whether the rheometer gives an adequate measure of 'spring', since the assessing of 'spring' by handling appears to be extremely difficult. It seems likely that when subjects are asked to judge 'spring', they tend to disagree as to the physical nature of the property which is meant, and quite different orders are given by different people, each order tending to be correlated with some definite physical property. The firmness orders tend to fall much more closely together than do the orders for 'spring'.

The ball compressor does not give such useful data as does the rheometer. It is not such an accurate instrument but is advantageous in that it is very simple to use and the cheese does not need to be cut. Again, we are at a disadvantage in that we have not yet been able to get a professionally made instrument.

Another expert was asked to grade twenty factory borings. The total deformations on the ball compressor were averaged for each group as assessed by the expert. The results are given in Table 6 and show a good correlation.

Table 6

Expert's score	9	8	7	6	5
Number of cheese	4	2	5	7	2
Average 'firmness'	0·293	0·227	0·197	0·183	0·159

The average 'firmness' is the reciprocal of the total deformation with the ball compressor.

Subsidiary experiment

It has often been noticed, especially with pasteurized milk, that it is not possible to make curd 'firm up' beyond a certain point either by continuing scalding or by increasing temperature. In the main experiment, for example, we had originally intended to pitch numbers ii and iv in the acid and 2 and 4 in the sweet experiments at considerably lower pitching points than was in fact possible in practice, even after prolonged scalding at rather a high temperature.

The subsidiary experiment showed marked differences in behaviour between the three cheese. The data are given in Fig. 1. The zero of the time scale is arbitrarily taken for a temperature of 85°.

It will be seen that rather excessive pasteurization has made a marked difference in the shape of the curve, the consistency increasing at first much more steeply with time; though the curd never became as firm as did the other two. The lightly pasteurized sample firmed up more than the control but softened again somewhat on further heating. This appears to be a usual characteristic of pasteurized milk curd. The consistency of the curd when heat was first applied was greatest in the case of the raw milk and least in that of the over-pasteurized; the latter thus had further to go in firming up than the others.

In all three experiments, temperature was raised at a steady rate, except that it was held approximately constant for a time at certain levels. It is striking how little effect these plateaux had on the shape of the consistency-time curve.

In general, the over-pasteurized curd showed a softer consistency for any given temperature than the others, the lightly pasteurized curd gave, possibly, a slightly greater consistency than that of the raw milk for most temperatures.

These results confirm quantitatively impressions already current among cheese makers about the effects of pasteurization on cheese making. It is hoped to extend these experiments so that our knowledge of these matters may be increased and made more precise.

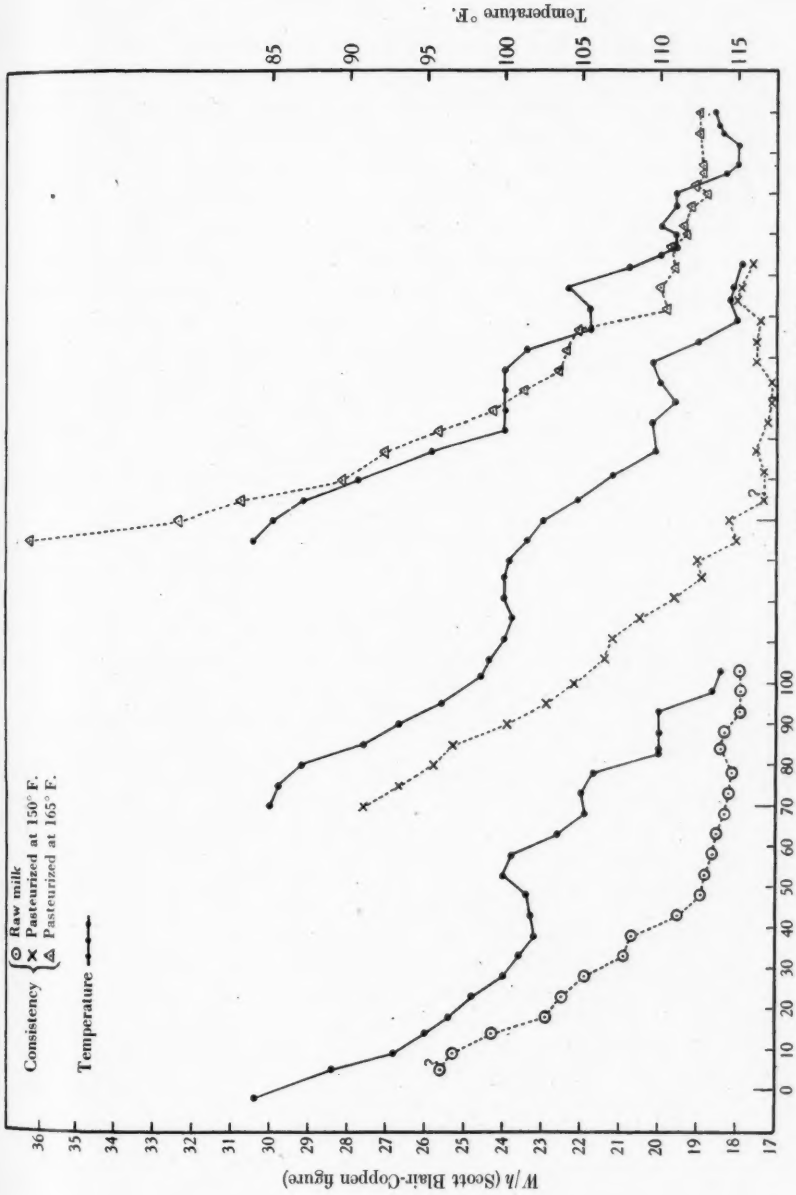
Consistency
Raw milk
Pasteurized at 150° F.
Pasteurized at 165° F.
Temperature
W/h (Scott Blair-Copper figure)
Temperature °F.
Time (mins.)

Fig. 1.

SUMMARY

1. Following the experiences gained from an earlier experiment, cheese were made at varying controlled pitching points and rates of scald, the experiment being done both with sweet milk and comparatively acid milk.

2. One of the two cheese made from each vat was submitted to physical tests at regular intervals during the ripening process and the other cheese, when ripe, were judged by a panel of experts who also, together with non-experts and routine analysts, gave judgements of firmness and 'spring'.

3. Tables were drawn up in which the rank orders of the cheese for each objective physical test and subjective judgement are compared with one another.

4. With the possible exception of vapour pressure and 'spring', the effect of pitching consistency was much more marked than that of the differences in rate of scald although the latter were considerable. Many physical properties were well correlated with pitching consistency.

5. The cheese makers are shown to have a greater self-consistency as individuals in picking out replicates and as a group in their decisions as to the rank order, by comparison with the other categories.

6. With the very sweet curd, most of the important properties of the cheese were highly correlated with one another and with the results of many of the physical tests. In this case, the difficulty which cheese makers usually experience in differentiating properties such as firmness from other quality factors did not arise, and they showed some superiority over the other categories. With acid curd there was little to choose between cheese experts and non-experts as far as correlation with physical tests was concerned, but routine analysts appeared to be somewhat better than either.

7. Conclusions are drawn as to the relative merits of various types of physical tests.

8. The rate at which the physical properties changed during the ripening process did not correlate with pitching consistency or with any other physical properties to any marked extent.

9. The effect of pasteurizing milk at various temperatures on the firmness of the curd during the scalding process has been measured.

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(*MS. received for publication* 6 *January* 1942)

637.333.1:541.18

297. THE FIRMNESS OF RENNET CURD; ITS MEASUREMENT AND VARIATIONS

By S. J. ROWLAND and D. SOULIDES, *National Institute for Research in Dairying, University of Reading*

(With 1 Figure)

There is a need for a simple reproducible objective test for the measurement of the firmness of the curd of small laboratory samples of milk coagulated under conditions approximating to those of the cheese-making process. In the investigations which have been made of the variation in the firmness of the rennet curd of different milks the firmness has in most cases been assessed by manual examination(1, 2, 3)—a procedure which has obvious limitations. Where objective methods have been used they have been open to criticism on the grounds that they were based on the measurement of the force required to cut the curd. Thus Sanders, Matheson & Burkey(2), appreciating the advantages of an objective test for curd firmness in the selection of milk for cheese making, applied Hill's(4) method for the measurement of curd tension to a rennet curd. Earlier, Alleman & Schmid(5) used a similar method. Recently Marquardt & Needham(6) have emphasized the need for a better method of evaluating the physical properties of the curd of milk samples with reference to the study of problems in cheese manufacture. They had investigated the use of the measurement of the curd tension of, surprisingly, a pepsin curd. Hill, however, originally introduced the measurement of curd tension to provide an index of the digestibility of a curd (for this purpose prepared with a calcium chloride-pepsin mixture as coagulant) and, as Scott Blair(7) has pointed out, it reveals the strength of the curd, i.e. its resistance to cutting, and neglects the physical properties related to firmness. The latter are not necessarily highly correlated with the former.

Scott Blair(7) has discussed the measurement of curd firmness and described an apparatus for the measurement of the elastic and plastic deformations, under a compressive load, of curd in the vat. He has shown that, at the stage when a normal curd in the vat is ready for cutting, its shear modulus is the best single criterion of its firmness. The same principle has now been applied to the measurement of the firmness of rennet curds prepared in the laboratory from 100 ml. samples of milk, and, after preliminary trials, a test has been evolved which is described in this paper. The utility of a test on these lines has been demonstrated by a series of experiments in which the test was used to investigate the variation in the curd firmness of different milks—with observations on the differences in composition involved—and the effect on curd firmness of variations in the following factors: acidity, addition of calcium chloride, amount of rennet, time of coagulation, and the admixture of soft-curd milk. Representative selections from the results are briefly described.

TEST FOR CURD FIRMNESS OF SMALL SAMPLES

Coagulation of the milk

The conditions of coagulation were chosen to simulate in a small-scale test the conditions of the cheese making (Cheddar) process; 0·02% of lactic acid was added to the milk, and a rennet concentration of 1 in 5000 used at a temperature of 32° C. for a coagulation time of 30 min.

One hundred ml. of milk were taken in a glass jar of 7·5 cm. diameter and 8 cm. height, and 1 ml. of 2% lactic acid solution was added. The jar was covered with a lid and placed in a thermostat at 32 ± 0·2° C., with the level of the water ½ in. above that of the milk, and was left for 20 min. to reach that temperature. Alternatively, the jar and its contents were quickly warmed to 32° C. The jar was then agitated gently whilst 1 ml. of a freshly diluted commercial rennet extract (2 ml. of rennet per 100 ml.) was added. The jar was

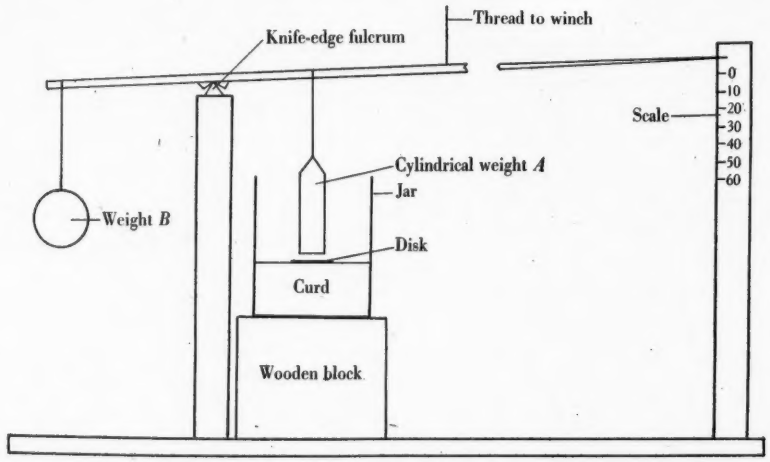

Fig. 1. Diagram of apparatus for measuring deformation of the curd.

then covered, and replaced in the thermostat for 30 min., when it was removed and the firmness of the curd measured immediately as described below.

Measurement of curd firmness

An apparatus consisting of a type of balance, as shown diagrammatically in Fig. 1, was assembled, for the measurement of the deformation of the curd under a compressive load. It was an experimental set-up from material available in the laboratory. A light disk of 2·5 cm. diameter was placed on the middle of the surface of the curd and a load of 6·0 g. applied to the disk through the weight *A*. This load was found to be the greatest which the softest homogeneous curds would bear without rupture. Preliminary tests with a series of curds ranging in firmness from very soft to very hard showed that the deformations produced, although varying widely in magnitude from sample to sample, were in all cases almost completely recoverable when the load was removed, the plastic deformation being negligible. The curds behaved as elastic gels of varying modulus, and since the jar may be considered large compared with the size of the disk, the shear

modulus n will be given approximately by the formula $n = F/8re$, where F is the force of compression, e the deformation observed, and r the radius of the disk on which the load was applied (7).

It was therefore possible to adopt, as a simple and reproducible measure of the firmness of the curd, the deformation produced under fixed conditions of load and time, thus obtaining a shear coefficient, a figure inversely proportional to the shear modulus. The determination was finally carried out as follows. The jar containing the curd was taken from the thermostat, the disk placed on the curd, and the jar placed below the weight A. By slowly turning a small winch carrying the thread T the balance arm was lowered until the weight A was just in contact with the disk and a reading (R_1) was taken on the millimetre scale. The thread T was then slackened, thus applying the load to the curd, and after 15 sec. a second reading (R_2) was made on the scale. The dimensions of the apparatus were such (from the fulcrum to the attachment of weight A to the arm was 6·0 cm., and to the scale 60·0 cm.) that the distance traversed by the pointer on the scale, i.e. $R_2 - R_1$, was 10 times the deformation of the surface of the curd. For convenience it was decided to record, as is done throughout this paper, the deformation of curds by whole numbers representing units of a tenth of a millimetre, these numbers being clearly the same as the scale distances $R_2 - R_1$ recorded in mm. Duplicate determinations of deformation made on separate 100 ml. samples of the same milk usually gave results which were either the same or differed by one unit.

THE VARIATION IN THE CURD FIRMNESS OF MILKS

One hundred samples of milk from forty-four individual cows were tested for curd firmness by the method described above. Most of the samples were taken during the months of June and August. The results are given in the first part of Table 1. Four of the samples (involving four different cows) either remained entirely liquid or formed a flocculent semi-liquid coagulum, and to these the measurement of deformation was obviously not applicable. For the samples which formed a homogeneous curd the deformations ranged from 12 to 30, with seventy-seven out of the hundred more or less evenly distributed over the range 17–25. The mean deformation, counting the uncoagulated samples at 30, was 21·8. A few herd samples of milk which were tested varied in deformation from 18 to 24.

The curd firmness of 125 separate quarter samples of milk taken during the winter period from thirty-two different cows was also determined. The results are given in the second part of Table 1. Again the largest deformation observed for a homogeneous curd was 30, the smallest deformation had decreased to 8. The mean deformation of this series was 19·3.

Data were available for the composition of these quarter samples of milk in terms of fat, total solids, casein, soluble protein, calcium and pH. As the cows comprised Shorthorns and Friesians in various stages of lactation, a wide range of composition was covered, as follows: solids-not-fat 7·35–10·11, casein 1·75–3·57, soluble protein 0·55–0·184, and calcium 0·079–0·196%, and pH 6·45–7·19. When the milk samples from the different quarters of the same cow were of practically uniform composition the deformations were in close agreement, as these examples of deformations per cow show: 29, 29, 29, 30; 19, 19, 19, 19; 17, 17, 17, 18; 13, 13, 13, 15; 12, 12, 13, 14; 8, 8, 10 (three-quartered).

When the samples of any cow varied appreciably in composition, showing the characteristic trend of quarter abnormality, namely a decreased casein, solids-not-fat and calcium content, and an increased soluble protein content and *p*H, the change in composition of the affected samples was reflected by a larger deformation; for example, 16, 16, 17, 21; 18, 19, 20, 23; 17, 17, 19, 27; 22, 23, 25, 30; 19, 21, semi-liquid, semi-liquid. When, however, the deformation and composition data as a whole were studied in the hope of assessing the quantitative relationship between the detailed composition of a milk and the firmness of its rennet curd, it became apparent that the problem was complex owing to the high degree of interaction between the various factors, and that much more data would be needed. For example, a high *p*H value corresponded with a medium, hard, or even very hard curd when the calcium and casein values were unusually high—as in some cases of advanced lactation—in spite of an accompanying extremely high soluble

Table 1. *Variation of the curd firmness of different samples*

Deformation	No. of samples	Deformation	No. of samples	Deformation	No. of samples
		Individual cow samples			
L*	4	24	9	17	6
30	1	23	6	16	2
29	1	22	11	15	4
28	2	21	8	14	4
27	2	20	12	13	1
26	1	19	5	12	1
25	9	18	11	—	—
		Separate quarter samples			
L*	2	23	7	15	8
30	2	22	6	14	4
29	3	21	9	13	5
28	—	20	10	12	1
27	2	19	26	11	—
26	1	18	12	10	1
25	4	17	12	9	—
24	2	16	6	8	2

* L = milk remained liquid or semi-liquid.

protein content, but corresponded with a very soft curd when the calcium and casein contents were normal or low. When the advanced lactation data were omitted there was, as would be expected, a general trend of increasing curd firmness with increasing casein and calcium values, and with decreasing soluble protein and *p*H values, taking these factors singly, but a wide range of deformation was observed for any given value. Thus for a casein content of 2·5% the deformation varied from 13 to 21; for a calcium content of 0·12%, from 16 to 25; for a *p*H of 6·5 from 13 to 23. Also there were indications that some factor in the composition, other than those which had been determined, was involved.

The curd firmness of a milk was found not to be affected by variations in its fat content. A number of measurements of deformation were made on whole milk, on the corresponding separated milk, and on separated milk plus cream giving milks containing up to 6·6% of fat. The deformations were the same for the whole and separated milks. When the physical character of a rennet curd is measured by its curd tension, however, the removal of the fat causes an appreciable increase in the rennet curd tension (2).

INFLUENCE OF VARIATION IN TREATMENT ON CURD FIRMNESS

Addition of lactic acid

To each of a series of milk samples covering a wide range of curd firmness 8% lactic acid solution was added in amounts varying from 0·25 to 1·50 ml. per 100 ml. of milk. Thus the lactic acid added varied from 0·02 to 0·12%. The *p*H of the milk-acid mixture was measured electrometrically, and the determination of curd firmness continued in the usual manner. Table 2 illustrates the type of results with data for three milk samples which, when treated with the routine 0·02% of lactic acid, failed to coagulate and showed deformations of 20 and 14 respectively. The *p*H values of the original milks were for A, 7·03; for B, 6·62; and for C, 6·45.

With some low *p*H milks the lactic acid was omitted and sodium hydroxide was added to raise the *p*H. To sample C, 0·5, 0·75 and 1·0 ml. *N*/2 NaOH was added per 100 ml. The resulting *p*H values were 6·59, 6·66, and 6·74; and the deformations 20, 22 and 'liquid' respectively.

The progressive change in deformation with change in *p*H, down to a minimum deformation at a *p*H of about 6·2 is clear. At any given *p*H the milks retain their differences

Table 2. *The influence of p*H *on curd firmness*

Lactic acid added (g./100 ml.)	Milk A		Milk B		Milk C	
	*p*H	Deformation	*p*H	Deformation	*p*H	Deformation
0·02	6·84	L	6·56	20	6·32	14
0·04	6·68	L	6·42	17	6·20	11
0·06	6·55	L	6·28	14	6·09	10
0·08	6·42	23	6·19	12	6·00	10
0·10	6·30	21	6·08	12	5·92	10
0·12	6·20	20	6·00	12	5·84	10

in firmness; *p*H adjustment alone will not reduce the deformation of milk A below 20, whereas the deformations of milks B and C, owing to differences in composition other than *p*H, have fallen to 12 and 10.

The addition of calcium chloride

To 100 ml. portions of each of a series of milks covering a wide range of curd firmness quantities of from 0·5 to 4·0 ml. of a calcium chloride solution containing 20 mg. Ca per ml. were added and the progressive increases in curd firmness observed. As the calcium chloride solution reduces the *p*H of the milk (on the average by about 0·13 per ml. added) the *p*H values of the milk-calcium chloride mixtures were determined, and in order to be able to dissociate the effect of the added calcium as such from the simultaneous effect of *p*H, aliquots of each milk without added calcium chloride were treated with increasing amounts of lactic acid (0·5–4·0 ml. of 2% lactic solution, each ml. of which, per 100 ml. of milk, also lowers the *p*H by about 0·13 on the average) and the deformations again determined.

It was found that when milk samples which form a normal or hard curd, as judged by the routine method, were treated with calcium chloride, the increase in firmness was entirely attributable to the *p*H change involved, but with some milk samples which

formed a softer curd the added calcium chloride increased the curd firmness more than did the amount of lactic acid necessary to reduce the *p*H to the same extent. For example, a sample of milk of *p*H 6·56 and a Ca content of 0·100% with the addition of 0·02% lactic acid gave a *p*H of 6·44 and a deformation of 25, but with no lactic acid and 20 mg. Ca per 100 ml. added as calcium chloride gave almost the same *p*H, namely, 6·42, and a deformation of 20.

Table 3 illustrates the same effect with soft-curd milk samples of high *p*H; sample D, with a *p*H of 6·80 and a Ca content of 0·132%, gave no coagulation by the routine method; and sample E, with a *p*H of 6·68 and a Ca content of 141 mg. per 100 ml., a deformation of 25.

Table 3. *The influence of calcium chloride on curd firmness*

Addition to milk (g./100 ml.)		Milk D		Milk E	
		*p*H	Deformation	*p*H	Deformation
Calcium added as $CaCl_2$:	0·02	6·68	28	6·56	17
	0·04	6·58	20	6·46	13
	0·06	6·46	15	6·33	11
	0·08	6·36	14	6·25	11
Lactic acid:	0·02	6·66	L	6·54	25
	0·04	6·58	24	6·48	19
	0·06	6·44	20	6·37	15
	0·08	6·36	18	6·27	14

The amount of calcium chloride added in these experiments was much above that used in cheese making, but the results for the larger additions have an academic interest in that the deformation decreased with increasing amounts of calcium chloride until limited by the *p*H effect at a *p*H of about 6·2. This observation is not in accord with Marquardt & Needham's(6) finding, when using the measurement of the curd tension of a pepsin curd in experiments intended to relate to problems in cheese manufacture, that a maximum hardening of curd is obtained with an addition of 0·08% calcium chloride—equivalent to 0·029% calcium.

Amount of rennet

A number of milk samples of varying curd firmness were coagulated with additions of rennet solution up to three times the normal quantity of 1 ml. of 2% rennet solution per 100 ml. of milk, i.e. 1 part of rennet to 5000 parts of milk, and deformations otherwise determined as usual. A typical selection of the results is given in Table 4.

Table 4. *The influence of the amount of rennet on curd firmness*

Rennet added (ml. 2% rennet sol. per 100 ml. milk)	Deformation of milk sample				
	F	G	H	I	J
1·0	L	26	24	20	15
1·5	26	24	22	18	14
2·0	23	22	20	17	13
3·0	21	21	20	17	13

Time of coagulation

The time which elapsed between the addition of the rennet and the measurement of the deformation, normally 30 min., was varied from 15 to 90 min. A selection of the results is given in Table 5. It is clear that the 30 min. period used in the routine test ensures a good differentiation between the firmness of the curds of different types of milk.

Table 5. *The effect of coagulation time on curd firmness*

Coagulation time (min.)	Deformation of sample						
	K	L	M	N	O	P	Q
15	23	25	29	L	L	L	L
20	18	20	23	25	L	L	L
25	16	18	20	23	26	27	L
30	15	18	20	21	23	25	L
40	15	17	18	20	22	23	L
60	12	15	17	18	20	23	26
90	12	11	17	16	18	21	22

The mixing of normal and soft-curd milks

Increasing proportions of soft-curd milk from an individual cow were added to normal bulk milk and the firmness of the mixtures determined. The soft-curd milk, which did not coagulate in the routine test, contained 2·11, 1·03 and 0·091% of casein, soluble protein, and calcium respectively, and had a *p*H of 7·04. The bulk milk contained 2·56, 0·54 and 0·128% respectively, and had a *p*H of 6·60. The results are given in Table 6. 5% of soft-curd milk was without effect on the firmness, and 10% increased the deformation only slightly (from 21 to 23), but 25% was sufficient to prevent the formation of a homogeneous curd.

Table 6. *Admixture of normal and soft-curd milks*

Percentage of soft-curd milk	*p*H of mixture at renneting	Deformation
0	6·46	21
2	6·48	21
5	6·50	21
10	6·52	23
15	6·52	25
20	6·54	27
25	6·55	L
30	6·58	L

DISCUSSION

The results of the investigation described indicate that a method of the type used provides a satisfactory measurement of the rennet curd firmness of small samples of milk. Such a method is of value for a number of purposes. It serves for the preliminary testing and classification on the basis of their curd firmness of supplies of milk to be used for cheese making, and in the case of supplies likely to form an undesirable curd under normal renneting conditions enables a pilot test to be made in which the effect on curd firmness of a modification in conditions of acidity, amount of rennet, etc., or the addition of normal milk or of calcium chloride, could be ascertained. It also facilitates the necessary further studies of the factors which influence curd firmness, in which connection the cause of occurrence and the peculiarities in composition of certain types of soft-curd milk(3) are particularly in need of investigation. There is a marked lack of quantitative data on the relationship between rennet curd firmness and detailed milk composition; the extensive literature on curd tension (see review by Doan(8)) is of no significance in this regard on account of the nature of the coagulant and of the method of testing.

The data presented in this paper should encourage the evolution, on the principle of the test used therein, of a standardized test for the rennet curd firmness of small samples of milk. The apparatus described in this paper was purely provisional, and a permanent

apparatus suited to the accurate measurement of small deformations of the curd should be designed. With agreement on, and specification of, details of the conditions of the coagulation and of the measurement of the deformation, a reproducible test for rennet curd firmness could be evolved which would fulfil the same function with regard to milk for cheese making as the measurement of curd tension, for which a standard method has been approved (9), fulfils with regard to the digestibility and suitability of milk for infant feeding.

Summary

A method is described for the measurement of the firmness of the rennet curd from 100 ml. samples of milk coagulated under conditions simulating those of the cheese-making process. Tests with a series of homogeneous curds ranging in firmness from very soft to very hard showed that the curds behaved as elastic gels of varying modulus, and the deformation produced under a suitable compressive load was accordingly adopted as a simple objective measure of firmness.

The method was used to observe the variation in the curd firmness of samples of milk from individual cows and separate quarters, with some observations on the relationship between curd firmness and milk composition, and the effect on curd firmness of variations in the following factors: acidity, addition of calcium chloride, amount of rennet, time of coagulation, and the admixture of soft-curd milk.

The results of these experiments indicate that the method provides a satisfactory measure of the rennet curd firmness of small samples of milk. The utility and applications of the method are discussed, and the suggestion is made that a standardized reproducible test on the same principle should be evolved to serve the same function with regard to milk for cheese making as the standardized measurement of curd tension serves with regard to the digestibility and suitability of milk for infant feeding.

The authors gratefully acknowledge their indebtedness to Dr G. W. Scott Blair for much help and advice.

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(*MS. received for publication* 6 *July* 1942)

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BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION C. DAIRY CHEMISTRY

I. Composition of milk:
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VII. Analysis of milk and milk products:
- (*a*) Milk
- (*b*) Butter and butterfat
- (*c*) Cheese
- (*d*) Miscellaneous

This review deals with the progress of dairy chemistry from the middle of 1939, that is, the end of the period dealt with in the previous review (1), to the beginning of 1942.

I. COMPOSITION OF MILK

(*a*) Cow's milk

The variations in the fat and solids-not-fat contents of milk have been studied by Caulfield *et al.* (2), who examined three-day composite samples of milk taken monthly from each of 26 Holstein, 26 Ayrshire, 15 Guernsey and 28 Jersey cows throughout a complete lactation period. They noted the variations in the fat and solids-not-fat contents due to breed, individuality within the breed, and season of the year. The following mean percentages of fat and solids-not-fat were obtained: Holstein 3·69, 8·52; Ayrshire 4·32, 8·91; Guernsey 5·09, 9·29; and Jersey 5·53, 9·54. Jacobsen & Wallis (3), similarly, investigated the effect of breed of cow, stage of lactation, and season of the year on the total solids, fat, ash, calcium, and phosphorus contents of one-day monthly samples of milk covering 60 lactation periods from 10 Holstein, 3 Ayrshire, 6 Guernsey, and 9 Jersey cows. Overman *et al.* (4) studied the composition of the milk of 17 Brown Swiss cows, covering 33 lactation periods. The mean results were solids-not-fat 9·40, fat 4·02, protein 3·61, lactose 5·04, and ash 0·73%, and specific gravity 1·0318. The bulletin gives a full statistical treatment of the data, including the variability and correlation coefficients of the constituents.

The effect of udder disease on the composition of milk continues to receive attention. Van Landingham *et al.* (5) investigated the relationship of the development of mastitis to changes in the chloride, lactose, and casein number of milk by noting the variations in the composition of the milk samples drawn from individual quarters of the same udder. Samples were obtained at intervals from 40 cows whose quarters were diagnosed as positive or negative on the basis of cultural and indirect tests, and these samples were classified into three groups. Group I included the quarter samples from udders in which all quarters were negative, group II the samples from negative quarters of udders in which one or more quarters were positive, and group III all the samples from positive quarters. The mean chloride contents of the samples in groups I, II and III were 0·124, 0·119 and 0·150% respectively, the mean lactose contents 4·79, 4·87 and 4·35%, and the corresponding mean casein numbers 77·6, 77·8 and 74·2. The

diagnosis of mastitis by comparing the composition of the milk samples from the different quarters of the udder is discussed at length. Shattock & Mattick (6) examined quarter milk samples from cows with subclinical staphylococcal mastitis; in most cases the presence of a significant number of staphylococci was accompanied by a decrease in the casein number and an increase in the *p*H. Rowland (7) has discussed the cause and significance of variations in the non-fatty solid constituents of milk, dealing particularly with the effect of mastitis on milk composition.

The effect of air raids on the fat and solids-not-fat contents of milk has been investigated by Rowland (8, 9). In war-time, in cases of prosecution for the sale of adulterated milk, the defence may on occasion be put forward that the cows which produced the milk samples concerned had been subjected to air-raid shock. Milk samples were taken from herds in various districts of southern England for a period after bombs had been dropped among or near the cows. In all cases it appeared that the composition of the milk had not been affected.

(*b*) Milk of other species

Golz (10) has studied the variations in the fat content of human milk. The fat content of 525 samples ranged from 0·5 to 10·0%, the mode being 3·5%. His data are grouped and discussed with regard to the effect of period of lactation, the difference between first and last milk drawn, the difference between the two breasts, and the age and health of the subject.

With increasing interest in the use of goat's milk, the composition of goat's milk has been the subject of further study. Lythgoe (11), and Trout (12) have recorded the composition of individual and herd samples of milk produced in Massachusetts and Michigan respectively. Besley (13) has compared the detailed composition of the milk of a Toggenburg herd of goats with that of a Holstein herd of cows, and found a close similarity.

Ghosh & Datta Roy (14) found the following ranges of composition for 231 samples of milk from individual buffaloes: specific gravity 1·028–1·040; and fat 2·0–12·0, solids-not-fat 8·5–15·5, and lactose 4·0–5·3%.

Anantakrishnan (15) has made a detailed study of the composition of the milk of the ass; his data show the general similarity between this and human milk. Kotschopoulos *et al.* (16) determined the composition throughout two lactations of samples of milk from six herds of sheep (each herd of a different breed) in Greece. Van Wagenen *et al.* (17) have given data for the composition of the milk of the monkey, Anderson *et al.* (18) of the dog, and Vos *et al.* (19) of the dolphin.

II. CHEMISTRY OF MILK CONSTITUENTS

(*a*) Milk fat

Hilditch and his associates continue to make valuable contributions to our knowledge of the composition of milk fat. Hilditch *et al.* (20) examined the distribution of fatty acids among the component glycerides of a typical milk fat from cows at summer pasture. The number of component acids in milk fat rendered impossible anything of the nature of an exact description of the many mixed glycerides undoubtedly present, but the general distribution of the four main groups of acids—oleic (including octadecadienoic and traces of unsaturated C_{20} and C_{22} acids), palmitic, stearic, and the lower acids of the C_4 to C_{14} series—are approximately indicated. The mixed glycerides, as per cent mol., were probably within the following limits: oleo-mono C_{4-14}-palmitins 31–22, oleo-palmito-stearins 8–17, palmito-dioleins 17–4, oleo-mono C_{4-14}-stearins 12–6, oleo-di C_{4-14} 4–9, mono C_{4-14}-dioleins nil–10, stearo-dioleins 8–1, oleo-dipalmitins 1–5, tri-oleins 0–7; and of the fully saturated glycerides, mono C_{4-14}-palmito-stearins 9, di C_{4-14}-palmitins 6–7, and small amounts of di C_{4-14}-stearins, mono C_{4-14}-dipalmitins, dipalmito-stearins, and palmito-distearins, amounting in all to 19%. The glyceride composition, with palmitic acid present in about 70% of the glyceride molecules, is similar to that of ox depot fat, with lower acids replacing some of the stearic acid. This supports the hypothesis that milk fat is the result of transformation in the mammary gland of pre-formed oleo-glycerides, including a large proportion of palmito-oleo-glycerides.

Hilditch & Jasperson (21) have compared the component fatty acids of a sample of butterfat from cows fed solely on silage and hay in winter with those of two samples of butterfat from cows on summer pasture. The composition of the fat from the cows receiving silage closely resembled that reported in earlier studies for the butterfat from cows given winter rations of hay, roots, and home-grown concentrates. The silage butterfat contained more myristic, palmitic and hexa-decenoic acid, and less stearic and oleic acid, than the pasture butterfats. Examination of the butterfats for the presence of polyethenoid C_{18} acids led to the conclusion that about 0·5% of the fatty acids was present as conjugated diethenoid C_{18} acid, thus confirming an earlier note on this subject (22).

Heiduschka & Cicekdagi (23) found the following composition for the fatty acids of the fat of Turkish buffalo milk: butyric 4·2, caproic 1·3, caprylic 0·4, capric a trace, lauric 3·0, myristic 7·2, palmitic 25·6, stearic 16·2, arachidic 3·2, oleic 35·2, and linoleic 2·0. These results are in agreement with previous data.

Hilditch & Maddison (24) have studied the component acids of the phosphatides present in cow's milk fat; two samples of phosphatides were examined, isolated from Swiss and English butterfat clarification residues. The component acids, expressed as percentage of the total phosphatide acids, were as follows: myristic 3·2, 5·5; palmitic 21·0, 13·4; stearic 7·3, 9·0; higher saturated 17·5, 30·9; hexadecenoic 4·3, 4·9; oleic 32·5, 23·5; octadecadienoic 6·4, 0·0; and C_{20-22} unsaturated 7·8, 12·8. Thus, although the two samples differed in some details, the data confirm the findings of previous investigators that the typical lower volatile acids of butterfat are absent from the phosphatides, and that the component acids of the phosphatides have little in common with those of butterfat glycerides. Kurtz & Holm (25) have examined the acids of the sphingomyelincerebroside fraction of the milk lipids, and found that about 80% consist of lignoceric acid and the remainder of a mixture of saturated and unsaturated acids from which only myristic acid, of which less than 1% was present, was isolated.

An interesting address surveying the chemistry, physics, and technology of the fat in milk has been given by Sharp (26) under the title 'The milk fat globule'.

(*b*) Proteins

From a solution of casein in ammonium chloride and sodium hydroxide Cherbuliez & Jeannerat (27), by the adjustment of *p*H and the addition of acetone, have separated the casein into three fractions, the yields of these being 32·8, 48·7 and 3·5% of the total casein. They consider the smallest fraction, which is not coagulated by rennet, to be identical with Hammarsten's old whey protein fraction, and assume that this is not a fission product of casein but an original constituent of the casein complex.

Hotchkiss (28) has determined the number of peptide bonds in Palmer's crystalline lactoglobulin. He titrated the carboxyl and amino and imino groups liberated by complete hydrolysis and calculated that the molecular weight of 39,000 derived from ultracentrifugal data represents 338 amino-acid residues. Sharp & Hand (29) have devised a method for determining free flavin and flavin combined as flavoprotein, and have found that about 0·07 g. of flavoprotein is adsorbed on 100 g. of fat as fat globules. Nicolet & Shinn (30), in disagreement with published findings, have not found any evidence of the presence of hydroxyglutamic acid in the milk proteins. They partition the total hydroxyamino acids between threonine and serine. Horn & Jones (31) have isolated lanthionine from lactalbumin.

Macara & Plimmer (32) have investigated the amino-acid composition of dephosphocaseose and found a general similarity with that of casein and dephosphocasein. On the assumption that each molecule of protein contains one molecule of cystine they calculate the following molecular weights: casein 83,300, dephosphocasein 80,000, and dephosphocaseose 100,000.

During the period under review three papers have appeared on the amino-acid composition of casein phosphopeptone (33, 34, 35). The contradictory data presented suggest that, although there is a certain general agreement about the composition of phosphopeptone, it must be realized that different conditions of preparation of this casein hydrolysate will lead to peptones of varying composition.

(*c*) Lactose

Mohr & Eysank (36) have studied the crystalline forms of lactose under the polarizing microscope. They describe and illustrate a number of different forms, some hitherto unknown, obtained under varying conditions of concentration, temperature, and velocity of crystallization.

(*d*) Mineral matter

Hutchinson (37) found the following mean values for 168 samples of milk collected over a period of a year in New South Wales, Victoria, and Tasmania: calcium 0·120, phosphorus 0·082, potassium 0·144, sodium 0·048, magnesium 0·012, and iron 0·000087%. The phosphorus content of the milk was low as compared with that reported in other countries, and he suggests that it is due to the low phosphorus content of the Australian pastures, fodder crops and soils. Hutchinson (38) has also studied the sulphur content of milk; for eight bulked milks this ranged from 0·029 to 0·034%. Data are given to illustrate that the sulphur content of a milk may be calculated from its casein, albumin and globulin contents or, approximately, by the equation: sulphur percentage = (ash percentage × 0·09) − 0·34.

Beck (39) has investigated the copper content of the milk of cows and ewes from normal and copper-deficient areas of Western Australia. The copper contents of the milk of cows in normal areas were mostly between 0·05 and 0·20 mg./l., whilst those of cows in deficient areas ranged from 0·01 to 0·05, and averaged 0·02 mg./l.

Garrett & Overman (40) determined at intervals the mineral composition of the colostra from an Ayrshire and a Holstein cow. Calcium, magnesium, sodium, phosphorus and chloride were high at parturition and during the early hours of lactation and then rapidly decreased; potassium was low at parturition and then gradually increased. Acharya & Devadatta (41) investigated the distribution of the compounds of phosphorus in buffalo milk.

(*e*) Enzymes

Lipase

Krukovsky & Sharp have continued their studies of milk lipase. They have confirmed that lipase is inactivated in the presence of heavy metals under conditions of low active oxygen concentration, and have shown that in the presence of dissolved copper inactivation is prevented by removal of dissolved oxygen, whilst in the absence of added copper there is greater lipolysis in deaerated than in normal milk (42). They have also dealt with the effect of the properties of the fat and of the fat globule surface on the rate of lipolysis (43).

Tarassuk & Richardson (44), dealing with the inhibition of lipase activity in raw milk, found that lipolysis does not take place while the milk is in the cow's udder. The lipase is activated by cooling the milk to 20° C. or below. They suggest that the cooling affects the permeability to lipase of the adsorption membrane around the fat globules, for agitation of milk, without cooling, also activates the enzyme. If milk is held at 32–37° C. for 1–3 hr. after drawing, subsequent lipolysis is much retarded.

Rennin

Struble & Sharp (45) have provided a series of data on the heat inactivation of rennin in whey under various conditions of temperature and *p*H. Bhima *et al.* (46), by dialysing an acid extract of calf mucosa and subjecting the resulting material to repeated extraction with acid and precipitation at *p*H 5·4, obtained a rennin with an activity and purity greater than have been reported hitherto. Its chemical nature is discussed. Berridge (47) has studied the second phase of rennin coagulation, that is the actual precipitation of calcium paracaseinate, and has found that the relationship between the logarithm of the rate at which the reaction proceeds and the temperature is linear. The temperature coefficient of the reaction was 1·3–1·6 per ° C., which suggests that the second phase is a denaturation of the casein.

Phosphatase

During the period under review a large number of papers has appeared on the phosphatase test for the control of pasteurization. Many of these papers contain little that is new, and need not be mentioned in this general review; they record the satisfactory use of the various modifications of the test or suggest minor modifications of technique. The following reviews have been published: 'The phosphatase test for control of efficiency of pasteurization', by Kay *et al.* (48); 'The phosphatase test. A review of the literature on its application for detecting irregularities in the pasteurization of milk and dairy products', by Burgwald (49); 'A critical review of the phosphatase test', by Burgwald (50); and 'The phosphatase test', a brief review, by Moloney (51).

Neave (52) has simplified Kay and Graham's test by using sodium hexametaphosphate to prevent the formation of a precipitate after the addition of the sodium carbonate, thus dispensing with the second filtration. The paper also deals with other essential points of technique, and discusses the effect of bacterial growth. Aschaffenburg & Neave (53) have adapted Scharer's rapid test for use in Great Britain with milk pasteurized at 145° F. for 30 min. and made a study of the factors influencing the reliability of the test, which has an incubation time of only 1 hr. Scharer (54) has made further observations on his rapid phosphatase test, and Gilcreas (55) has reported the results of a collaborative study, undertaken by a number of laboratories, of the various modifications of the phosphatase test. The accuracy of the Gilcreas and Davis modification was confirmed, and the adoption of this tentative method as an official method was recommended. Scharer's laboratory method was found to be of similar accuracy, and was proposed as a tentative method. Scharer's field test was considered to be adequate for routine control; it should not be used in place of the more sensitive methods for accurate laboratory work.

Wiley *et al.* (56) have made a valuable study of some factors influencing the phosphatase reaction of flash pasteurized cream and of butter made from it. Cream pasteurized at a temperature of about 200° F. in a Vacreator, and giving a low phosphatase value, yields a butter giving an unexpectedly high phosphatase value in the serum. This is apparently due to the binding of a small proportion of the enzyme in the cream in such a way that it escapes destruction during the short heat treatment. The bound phosphatase is liberated by treatment of the cream with salt; hence the high value given by the salted butter. The phosphatase test therefore cannot be used on the butter to indicate whether the cream has been properly flash pasteurized.

(*f*) Other constituents

Using the term lecithin to designate the fat-like substances containing phosphorus that are extracted from milk and its products by the Mojonnier alcohol-ether method, Heinemann (57) found the following lecithin percentages: skim milk 0·015–0·018, raw whole milk 0·035–0·036, skim milk from reseparated cream 0·035–0·093, pasteurized sweet cream 0·006–0·199, buttermilk 0·114–0·126, butter 0·153–0·212, and separator slime 0·229. From these data he discusses the relationship of lecithin to fat.

Heller & Swiechowska (58) determined the ammonia content of over 600 samples of fresh milk and market milk. The ammonia content of fresh milk varied from 0·30 to 0·93 mg. of ammonia N per l., and averaged 0·64 mg. Data are given showing the marked increase in this figure for market milk and milk stored at various temperatures. The determination of ammonia is considered of value as a guide to the hygienic quality of milk. Noll & Supplee (59) have investigated the factors which influence the gas content of milk. The gas content of 63 samples of the mixed raw milk of a commercial depot was: oxygen 0·30–0·59, mean 0·47; nitrogen 0·18–1·63, mean 1·29; and carbon dioxide 3·44–6·28, mean 4·45 vol. %. Milk was deoxygenated by subjecting to vacuum treatment, flushing with other gases, or adding ascorbic acid and exposing to light.

III. MILK PRODUCTS

(*a*) Cream

Wiley & Newman (60) have discussed the neutralization of sour cream for buttermaking. They deal with the estimation of the acidity of cream, and give data to show the relationship between the titratable acidity and *p*H of raw cream, of neutralized pasteurized cream, and of buttermilk, and to show the relationship between the *p*H of butter and buttermilk and the cream from which they are made. The closeness with which one can hope to control the *p*H of butter by adjusting the titratable acidity of the cream is illustrated. Newman & Wiley (61) have continued the subject by discussing some factors affecting the accuracy of neutralization.

(*b*) Butter

Prill *et al.* (62) have reviewed the literature on the occurrence and properties of diacetyl, its higher homologues, and other α-dicarbonyl compounds, with special reference to the flavour of butter, and have prepared a number of these compounds. Dilute aqueous solutions of acetyl propionyl, acetyl *n*-butyryl, dipropionyl, etc., had odours like diacetyl but weaker; whereas the α-dicarbonyl compounds other than the higher homologues, e.g. α-keto-aldehydes and alicyclic and aromatic diketones, differed distinctly from diacetyl in odour and other respects. When acetyl propionyl or dipropionyl were worked into salted butter the flavour definitely suggested diacetyl, but was less than that of an equivalent amount of diacetyl. Distillates from ordinary butter cultures did not contain higher homologues of either diacetyl or acetylmethylcarbinol. Prill & Hammer (63) have observed the changes in the diacetyl and acetylmethylcarbinol contents of butter kept at various temperatures.

Herzer *et al.* (64) have studied the effect of various feeds on the fat constants, heat resistance, flavour and texture of Mississippi butter, and have compared these characteristics for samples of Mississippi and Minnesota butter over a period of a year. Much of the butter made in the cotton belt is firmer than that made in the northern states, more resistant to heat, gummy, and of a flat oily flavour; it therefore receives a lower score on the northern markets. It is concluded that the feeding of cotton-seed products, the use of dry hays as roughage, and the predominance of the Jersey breed, are the most probable factors responsible for the hard texture of the Mississippi butter.

Vollhase & Rauschning (65), on the basis of a large series of observations of the order, taste, appearance, grading score, and the results of chemical testing, of a variety of butter samples, point out that the safest method by which to judge the quality of butter is sensory grading and not chemical testing.

An account of the composition and deterioration of butter has been given by Kretchmar (66).

(*c*) Cheese

Nicholls (67) has given a series of correlation tables and regression equations for the water and fat contents of several varieties of English cheese examined between 1933 and 1939. The mean percentages of water and of fat in the cheese, and of fat in the dry matter, in this order, were as follows for each variety, with the number of samples given last in brackets: Cheshire 40·53, 29·96, 50·30 (606); Cheddar 36·69, 31·82, 50·20 (363); Lancashire 44·28, 27·39, 49·10 (403); Stilton (white) 41·76, 32·44, 55·37 (124); Stilton (blue) 37·64, 35·71, 57·20 (212); Caerphilly 44·67, 28·43, 51·23 (234); Wensleydale 45·03, 27·72, 50·23 (111); Leicester 39·79, 30·21, 50·00 (24); Derby 39·95, 30·59, 50·7 (11); and Cream 24·50, 71·60, — (49). The following formula is deduced for application to cheese from milk or cream:

$$W + mF = \frac{100 - S}{100} K,$$

where W, F and S are the percentages of water, fat and salt in the cheese, m is a factor dependent on the percentage of fat in the original milk, and K is a constant equal to 93·3 for cream cheese and 93·0 for pressed cheese. The paper gives details of the derivation of the

formula and its use in estimating the percentage of fat in the original milk or cream. The factor *m* is regarded as a slightly better criterion than the percentage of fat in the dry matter of a cheese for determining whether the cheese was made from whole milk.

Marquardt & Yale (68) have given details of the fat, moisture, and salt contents of the Cheddar cheese exhibited at the 1940 and 1941 New York State fairs, and of a number of Cheddar cheese selected over a wide area of the State. Buckles (69) has determined the fat and moisture contents of a few samples of each of several varieties of cheese. Dern & Dahlberg (70) identified the white particles about the size of a small pin head which form in and on the surface of the parchment paper wrapping around canned Cheddar cheese as consisting almost entirely of tyrosine. In view of the earlier evidence of McDowall and McDowell that the white particles found throughout the body and in the cracks of mature Cheddar cheese consist largely of calcium lactate, it therefore appears that more than one type of particle is involved.

Continuing their studies on the chemistry of cheese-making Dolby *et al.* (71) have now dealt with the measurement of the acidity of cheese and the relation of acidity to grading score. They find the *p*H of Cheddar cheese when it is 14 days old to be the most useful means of measuring the extent of acid development in the cheese and to show a very fair agreement with the observations of the graders on the mature cheese. Cheese with the *p*H value close to 4·90 at 14 days gained the highest average score at maturity. The *p*H or titratable acidity values for mature cheese are less useful indicators of the quality of the mature cheese than are the values at 14 days.

Davis (72) has given a general account of the significance and source of enzymes in cheese, and Hiscox (73) has discussed the development and measurement of the volatile acids of cheese and the differences in content and distribution of the volatile acids of different types of cheese.

(*d*) Other products

The composition of the ash of dried skim milk and its relation to neutralization have been studied by Hillig (74). Sodium lactate and calcium lactate were added to a reconstituted unneutralized dried skim milk and the effects on the alkalinity of the total and water-soluble ash and on the distribution of sodium and potassium between the water-soluble ash and water-insoluble ash were determined. When sodium lactate was added its alkalinity was only partly returned as alkalinity of the water-soluble ash, its sodium was found partly in the water-insoluble ash, and the proportion of potassium in the water-soluble ash had increased. When calcium lactate was added there was an unexpected increase in the alkalinity of the water-soluble ash, calcium having replaced most of the sodium and all of the potassium in the insoluble ash. The increase in the alkalinity of the water-soluble ash is therefore not a measure of added neutralizers as unexplained base-exchanges take place.

The British Standards Institution (75) has issued a specification for pure vacuum salt for dairy purposes. The salt shall be white, give a clear colourless solution, contain not more than 0·20 and 4·0% of moisture in dried and undried salt respectively, and contain not less than 99·7% of NaCl on a moisture-free basis. Dried salt as packed shall pass completely through an 18-mesh British standard sieve. The following percentage limits of impurities are specified: alkalinity 0·03 as Na_2CO_3, matter insoluble in water 0·03, sulphate 0·10 as Na_2SO_4, iron 0·002, copper 0·0002, arsenic 0·0001, lead 0·0005; and calcium and magnesium shall conform to the tests prescribed. Methods of sampling and analysis are stipulated.

Doctor *et al.* (76) have investigated the effect of the method of preparation, the breed of animal, and the feeding of various oil-cakes on the grading and physical and chemical constants of ghee; and Narasimhamurty (77) has observed the effect of ageing in the presence of light and air on its fat constants. A second edition has been published of Godbole & Sadgopal's (78) book entitled *Butterfat (Ghee)*. It is several times larger than the first edition and deals with composition, nutritive value, digestibility, rancidity, adulteration, detection and estimation.

IV. PROCESSING AND MANUFACTURE

Holland & Dahlberg (79, 80) have made an extensive study of the effect of time and temperature of pasteurization on the creaming power, phosphatase test, bacterial destruction, rennet coagulation, calcium phosphates and nitrogen partition of milk. The heating was accomplished in a thin-walled rectangular tinned copper tank of dimensions $20 \times 12 \times 1$ cm., and the milk was held for various times at temperatures ranging from 140 to 175° F. Haller *et al.* (81) have observed the effect of pasteurization on the soluble calcium, phosphorus and protein contents and on the curd tension and phosphatase of goat's milk. A series of two quart samples of the milk was pasteurized in the laboratory at 160° F. for 15 sec. and at 142, 145 and 147° F. for 30 min. The phosphatase test as normally conducted is not applicable to goat's milk; the enzyme is inactivated sufficiently for the milk to pass this test when it has been heated for only 5 min. at 143° F.

Miller & Sommer (82) have investigated the effect of *p*H, salts, evaporation, and previous heat treatment on the coagulation temperature of milk, using small samples of skim milk sealed in 8 mm. glass tubes. The coagulation temperature was at a maximum at *p*H 6·4–6·5. The addition of disodium hydrogen phosphate, sodium citrate, or potassium carbonate gave greater heat stability except at *p*H values above 6·5. The addition of calcium chloride or citrate reduced heat stability at *p*H values below 6·5. Pre-heated and concentrated skim milk also show maximum stability at *p*H 6·5; and the addition of salts had the same effect as in the original milk.

As a means of elucidating the chemical factors involved in the browning of autoclaved milk, Kass & Palmer (83) studied the chemical changes which characterize and accompany the caramelization of lactose by following the development of colour and the reducing, optical, and acid-forming properties of lactose solutions heated in the presence of various inorganic and organic buffers, including mixtures of milk salts, and caseinate sols, over extended ranges of *p*H, concentration, and time. The trend of the caramelization reaction was the same for all buffers, and the development of coloration was a logarithmic function of the optically inactivated lactose and directly proportional to the initial concentration of lactose. Sodium caseinate sols affected the lactose like other buffers, and casein adsorbed caramel according to the Freundlich isotherm. Kass & Palmer concluded that the brown coloration might be accounted for on the basis of the caramelization of lactose by the casein and the adsorption of the lacto-caramel by the caseinates, as distinct from the usual postulation of the formation of a casein-lactose condensate involving amino groups.

The effect of processing on the nitrogen distribution in milk has been investigated by Menefee *et al.* (84) who used Rowland's procedure to determine the total, protein, casein, albumin, globulin, proteose, and non-protein nitrogen fractions in raw, pasteurized, homogenized, evaporated, cultured, and enzyme-treated milks. As would be expected, the albumin and globulin in the evaporated milk was denatured and co-precipitated with casein, and there was slight proteolysis. The cultured and enzyme-treated milks showed slight and marked proteolysis respectively.

V. DEFECTS OF MILK AND MILK PRODUCTS

The effects of the various factors involved in the production of oxidative off-flavours in milk and its products have been extensively reviewed up to 1940 by Brown & Thurston (85). The compounds formed during oxidative deterioration and the theories concerning their formation, the methods of predicting and detecting the onset of oxidation, and the action of antioxidants are also discussed. Oxidized flavour has been the subject of many papers during the period since the previous biennial review. These papers have dealt mainly with the effect of the feed of the cow and with the use of various antioxidants; few have contributed to our understanding of the actual mechanism of the development of the off-flavour.

Direct evidence in support of the theory that the oxidation involves the phospholipid fraction of the fat has been obtained by Swanson & Sommer (86). Milk and cream to which 3 p.p.m. of copper as copper sulphate had been added were held at 4° C. for 3 days to develop oxidized flavour. The milk was then separated, the creams were churned, the buttermilks extracted by

the Röse-Gottlieb method, and the phospholipids precipitated with acetone. Unoxidized control samples of milk and cream were treated similarly. The iodine values of the phospholipid fraction of the oxidized milk and cream were 33·5 and 33·7 respectively, and the corresponding values for the control samples 60·3 and 48·7. The iodine values of the butterfat of the oxidized and control samples were not significantly different. Nelson & Dahle (87) also found no significant change in the iodine value or peroxide number of the butterfat isolated from samples of milk and cream which had developed a pronounced oxidized flavour on storage at 4·5° C. for periods up to 6 weeks. The same result was observed by Ritter & Nussbaumer (88).

The study of relation of the oxidation-reduction potential to the development of oxidized flavour has been continued. Swanson & Sommer (89) found that the addition of copper sulphate and of ferric sulphate to milk increased the potential, 50 p.p.m. of ferric iron having about the same effect as 0·25 p.p.m. of copper. The addition of ferrous sulphate steadily decreased the potential, yet a sample of milk containing 50 p.p.m. of ferrous iron developed a more intense oxidized flavour than did a corresponding sample containing 5 p.p.m. of copper, although there was a difference of 0·135 in the E_h. It appeared that the development of oxidized flavour is dependent on the catalytic action of the metals and not on the changes in potential which they cause. The potential of milk was also lowered by passing an electric current through the milk, but the off-flavour development was not inhibited. The conclusion was reached that the oxidation-reduction potential of the milk seems not to affect the development of metal-induced oxidized flavour. Greenbank (90) has reached a different conclusion in a paper which deals with a number of the factors concerned in the development of oxidized flavour in milk but gives special consideration to the variation in the oxidation-reduction potential as a cause of oxidized flavour. His data, which are representative of the results obtained from the examination of over 4000 samples of milk from about 300 cows, are presented under the following headings: the detection of samples susceptible to flavour development, the cause of variations in the susceptibility of individual milks, the promotion and inhibition of oxidized flavour by oxidizing agents, the control of the development of flavour by variations in the potential, and the effect of metals, feeds, storage temperature, heating, bacterial growth, air, light, antioxidants, and variation in ascorbic acid content. The data indicate that these various factors affect the development of oxidized flavour through their effect on the E_h of the milk, and that milk which develops the flavour spontaneously may be very poorly poised, milk susceptible to development of the flavour on the addition of copper poorly poised, and non-susceptible milk well poised. Larsen *et al.* (91) have demonstrated that the homogenization of milk has no effect on its oxidation-reduction potential or on subsequent changes therein. Hence the mechanism by which homogenization prevents or retards the development of oxidized flavour appears not to be associated with the potential.

Gould (92) found that the heating of milk momentarily at temperatures of 80–82° C. or above (about 15 min. being taken to reach these temperatures) prevented the development of oxidized flavour. It produced a cooked flavour and the liberation of sulphide, and the view is held that the reducing system which is formed when milk is heated is responsible for the retardation or prevention of oxidized flavour development. Gjessing & Trout (93) investigated the effect of various heat treatments of milk upon the stability of ascorbic acid and upon the development of oxidized flavour. When samples of mixed milk were heated for 10 min. at 65, 70, 75, 80, 85 and 90° C., heating at 80° C. had the greatest stabilizing effect. Corbett & Tracy (94) found that the development of oxidized flavour in condensed whole milk and in milk reconstituted from it was prevented by condensing under vacuum to a concentration of 2 to 1. The same effect was obtained by condensing skim milk and remaking 4% fat milk from cream, condensed skim milk, and water. As the results indicated that more than the simple removal of oxygen was involved, additional studies are being made concerning the possible liberation of antioxidative or reducing substances from a non-fatty solids constituent, particularly the protein. A series of papers by Sharp *et al.* (95, 96, 97) has described preliminary work on the deaeration of market milk as a means of retarding the development of oxidized flavour and the destruction of ascorbic acid.

Garrett (98) has investigated the interrelation of various metals and their salts on the development of oxidized flavour. Manganese greatly retarded or completely prevented the development

of the flavour in milk containing ferrous iron or copper and in milk which was spontaneously susceptible. It also checked further development of flavour which had already appeared. Henderson & Roadhouse (99) have studied the influence of various 'white-metal' copper-nickel alloys on the flavour of milk. Alloys which contained tin and zinc showed the least effect and the lowest corrosion rate.

With regard to the effect of the feed given to the cow on the development of oxidized flavour in the milk, Corbett & Tracy (100) and Brown *et al.* (101) have fed coconut, corn, and soybean oils and noted the changes in the iodine value of the butterfat and the development of flavour. The results were not consistent. With control cows on rations of low fat content the small variations in the iodine value of the butterfats were not correlated with the intensity of the oxidized flavour developed (101). Brown *et al.* (102) found that the feeding of high quality green alfalfa hay (carotene content 43·0 mg./kg.) and leaf meal to cows producing milk susceptible to the development of copper-induced oxidized flavour greatly reduced or eliminated the flavour development, whereas the feeding of low quality brown alfalfa hay (carotene content 5·8 mg./kg.), although it decreased the carotene content of the butterfat, did not increase the intensity of the flavour. No relationship was found between the carotene content and the intensity of the induced oxidized flavour of the milk of a number of cows on a normal dry feed ration. It appears that some substance associated with carotene in green alfalfa hay may be more important than carotene itself in reducing the susceptibility of the milk. Gould *et al.* (103) experimented with five different rations, finding that a pasture supplement reduced the incidence of oxidized flavour, and Garrett *et al.* (104) compared the effects of seven types of roughages.

The trial of cereal flours and their extracts as antioxidants in milk and dairy products continues to be reported (105, 106). Garrett (107) and England & Wiedemer (108) have investigated the use of oat flour-treated milk cartons, for the direct addition of oat flour to milk produces an undesirable, although not unpleasant, oat flour flavour. Combs *et al.* (109) have investigated the use of avenized parchment for wrapping butter. Dahle & Nelson (110) have attempted to determine the active antioxidant fractions of oat and soya-bean flour, and Nelson & Dahle (111) have presented a series of data which shows that ascorbic acid, oat flour, trypsin and steapsin preparations, gum arabic, and crude sugar inhibited the development of copper-induced oxidized flavour in pasteurized milk, and that citric acid, pepsin and refined sugar did not. Corbett & Tracy (112) found that the addition of 0·02–0·04% of tyrosine and its esters to milk, and of one part of pancreatic extract to 25,000 parts of milk, prevented the development of copper-induced oxidized flavour, and that a concentrated water extract of oat flour delayed it. This extract, or avenized salt, retarded the development of oxidized flavours in butter. Earlier, Anderson (113) described the effective commercial use of pancreatic enzyme to prevent the development of oxidized flavour: 1 g. of the powder was sufficient for 10–40 gal. of milk, according to the intensity of the flavour.

Waite (114) has investigated the use of several concentrations of oat flour and hydroquinone to delay the development of tallowy flavour in milk powder. The addition of 0·1% of oat flour to the original milk was ineffective, but 0·25% added to the milk or 2·5% to the powder delayed the onset of tallowiness, but not of cardboard flavour, by the equivalent of 4 months' normal storage. Hydroquinone proved completely effective, but is excluded on account of metallic flavours and legal objection. Govindarajan & Banerjee (115) have studied the protective action of various antioxidants in ghee. Kamala dye, which is harmless, odourless, stable, and of vegetable origin, was the most effective.

The source of the activated flavour which is acquired by milk exposed to ultra-violet radiation has been studied by Flake *et al.* (116). Irradiated casein and albumin possessed the typical odour and flavour of milk so treated, and irradiated cystine, methionine, tryptophane and histidine acquired strong flavours. Flake *et al.* steam-distilled intensely irradiated casein and prepared a concentrate which had a very strong odour typical of excessively irradiated milk and which imparted to milk a flavour similar to that of milk exposed to radiation. The concentrate gave a positive test for disulphides: these were probably formed from sulphydryls during the isolation of the concentrate. Milk samples which had been exposed to ultra-violet radiation contained more sulphydryl compounds than milk samples not exposed, and their sulphur was more heat labile. Sulphur compounds from the protein apparently have a role in the production of activated flavour.

There is evidence that the formation of sulphydryl compounds is the cause of the cooked flavour which results when milk is heated to a sufficiently high temperature (117, 118). The formation of these compounds, which are active antioxidants, serves to explain the lowering of the oxidation-reduction potential, and the decrease of the rate of the development of oxidized flavour and of the destruction of ascorbic acid, which occur when milk is heated above about 170° F. Gould & Sommer (117) considered that the liberation of sulphur compounds is due largely to a substance which is associated with the fat globules and passes into the buttermilk when cream is churned. Josephson & Doan's (118) experiments pointed mainly to lactalbumin as the source of these compounds, with the protein constituent of the fat globule membrane as a possible additional source. Gould (119) has reviewed the knowledge of the cause and prevention of cooked flavour in milk.

Mohr & Arbes (120) have discussed the development of fishiness in butter. They observed that the fishy taste produced by the addition of trimethylamine to butter was of a distinctly different type from that produced in butter on storage. A procedure was evolved which permitted an approximately quantitative recovery of the added trimethylamine without decomposing the lecithin. When this procedure was applied to a number of naturally fishy butters trimethylamine could not be detected. In agreement with a number of other workers on this subject, Mohr & Arbes concluded that the substance which gives butter a fishy taste is not always trimethylamine, and they suggest other possibilities.

VI. PHYSICAL CHEMISTRY AND PHYSICS

Remarkably few papers have been received during the period under review.

(*a*) Milk and general

Mathews *et al.* (121) found that the activities of dilute solutions of pepsin and of rennin in water decreased with pressure and length of time exposure at constant temperature. The loss of activity appeared to be the result of protein denaturation by pressure. Chemically, the product of pressure denaturation seems similar to that produced by heat; physically, there were marked differences.

Sell (122) studied the flow of milk through pasteurizers. His treatment is applicable to streamline conditions, whereas the flow is, as the author points out, in general turbulent. Sharp & Krukovsky (123) have shown that the surface tension of skim milk, cream and milk reconstituted from these varies with the temperature of separation.

(*b*) Curd and cheese

King & Melville (124) report a modification in the design of the viscometer previously described (vide last Review) for the study of the setting of milk into curd. Roundy & Price (125) describe a penetrometer in which a cone of standard size and shape is forced into the body of cream cheese in a beaker. The pressure exerted is recorded on a spring balance from which the beaker is suspended. The weight rests on a piston sliding in a cylinder which is by-passed so that the air displacement, and hence the rate of fall of piston and weight, can be controlled by means of a valve. When the valve is closed, the load of the piston and weight is borne by the cushion of air. The optimum dimensions for the apparatus are discussed and a few experimental data are also given.

Koestler, whose earlier work was mentioned in the last Review, has published (126) a series of papers on the rheology of Emmental cheese. His method, described in the first paper, is to load blocks of cheese 6·5 × 6·5 × 2·5 mm. in size for 2 min., measuring the compression at 15 sec. intervals and then to record the rate of recovery during the subsequent 2 min. The loads used are 50, 100 and 200 g.; most frequently the last. There appears to be an algebraic slip in his calculation of the elastic component of strain which is repeated throughout the work and partially vitiates his conclusions. The elasticity is considered to be dependent on the calcium phospho-caseinate structure. Very peculiar curves are given relating the percentage of deforma-

tion recovered elastically to the load. Increase in salt content decreases the total deformations without regularly affecting the elastic recovery. Fairly wide variations in moisture content do not affect elastic recovery, nor does the age of the cheese between 7 and 11 months. Variations in elasticity in samples from different parts of the same cheese cannot be paralleled by differences in chemical composition. Koestler (127) has also studied the flattening out (*Einfallen*) of Emmental cheese but finds no correlation between this phenomenon and rheological or analytical data. He concludes that it is controlled by variations in proportion of free and bound water. In the third paper (128) he considers two fundamental rheological properties of cheese. Hardness-softness and structure (*zäh-fein*). There are thus four possible extreme types, with normal cheese as intermediate varieties. The fourth paper (129) deals with the swelling capacity of cheese in distilled water and in salt solutions of various concentrations, which is correlated with cheese quality and especially with the physical condition of the proteins. Another series of papers by the same author (130) covers much the same ground.

Scott Blair *et al.* have developed a method for measuring the consistency of cheese curd at the pitching point. A sample of curd is collected in a cylindrical sieve from which it is cast into a solid cylinder, the weight and height of which are measured. Scott Blair & Coppen (131) show that the ratio of this weight to height (the 'pitching number') which is essentially a measure of superficial density, forms a good criterion of the consistency of curd for pitching. In further studies, Scott Blair & Coppen (132) and Scott Blair *et al.* (133) have studied the effects of systematic variations in 'pitching numbers' on the quality of the finished cheese as judged by rheological tests as well as by expert cheese-makers and others. A comparison is made between the skill of the cheese-maker and that of the baker who, having no special qualification to judge cheese, is nevertheless used to judging the quality of a not dissimilar material (dough) by handling. Scott Blair & Coppen (134) and Coppen (135) have followed up the psychological aspects of the judging of 'body', firmness, etc., of cheese-like materials by handling. They also describe an apparatus (132) used in many of the later investigations, in which a metal sphere is pressed into the surface of a cheese with a measured force. The deformations, immediate and permanent, are separately recorded. The exactitude of timing the pitching test is a disadvantage in a busy factory and Scott Blair & Scott Blair (136) have shown that, provided that the approximate proportions are maintained, the careful timing originally believed essential is unnecessary.

Davis & Scott Blair (137) summarize the test in a suggested method for keeping cheese manufacturing records, which also includes records of *p*H, titratable acidity, temperature, moisture and protein breakdown.

At a symposium held under the auspices of the Food Group of the Society of Chemical Industry and the Society of Public Analysts (138) Kay introduced the discussion by summarizing some recent chemical and physico-chemical findings, and Scott Blair summarized recent rheological work. In order to study the colloidal changes taking place in ripening cheese, Scott Blair *et al.* (139) have devised a simple method for measuring the vapour pressure of cheese. A similar method is also applicable to milk.

(*c*) Cream

Palmer & Tarassuk (140) have continued their studies on the physical and chemical properties of the fat globule adsorption membrane, and discuss the nature and origin of surface active materials involved in curd tension reduction and prevention of rennet clot in cow's milk by the addition of 'membranes' from natural and synthetic creams. Skelton & Herreid (141) measured the viscosity of creams in an orifice viscometer (the 'Borden body flow meter'). Comparisons were made between normal and washed, and pasteurized and unpasteurized creams. For unpasteurized cream, temperature treatments were found to be more effective in winter than in summer. For pasteurized cream, seasonal differences were apparently slight. Wiese *et al.* (142) discuss the improvements in viscosity of pasteurized and unpasteurized creams by heating and cooling, a technique which is given the rather unhappy name of 'rebodying'. The authors discuss which constituent of milk makes cream 'rebodiable' and decide that it is mainly the membrane on the fat globules. Skim milk does not stabilize reconstituted cream so that it can be 'rebodied',

but there are compounds in the aqueous phase which help in the process. Scott Blair *et al.* (143) study the very complex flow phenomena when cream is caused to flow through glass capillaries. Skelton & Sommer (144) find the capillary viscometer unsatisfactory for thick cream and prefer the Gardner Mobilometer in which a disk is allowed to sink through the cream in a cylinder. They criticize conclusions about fat-globule clustering, reached from microscopic examination, since the clusters are broken down in the dilution needed to prepare the sample for the slide. The practical object of this work is to clarify the complex phenomena associated with rise in viscosity induced by alternately cooling, warming and cooling pasteurized cream or by agitating the cream during cooling.

(*d*) Ice cream

Penczek & Dahlberg (145) study the false body of ice-cream mixes with a view to finding the best method of breaking down the mix so as to get a reproducible viscosity in an Ostwald viscometer ('Basic viscosity'). Appreciable resetting takes place if the stirred material is left for more than 5 min. Hening's ideas on cream structure are also discussed.

Erb (146) studies the viscosities of chocolate coatings of ice-cream bars using a MacMichael viscometer. The effects of lecithin and of varying moisture contents are investigated. An empirical test is also used (the 'Freundlich Bob-test') in which a metal bob is lowered into the chocolate, withdrawn, and allowed to drain under standard conditions, the amount of chocolate retained after draining being determined by weighing.

(*e*) Butter

Interruption of communications with Holland has made it impossible to report on work from the principal centre in which butter rheology was studied. Mohr (147) discusses the structure of butter, the influence of differences in temperature treatment and working and the effect of seeding with small crystals of butterfat on firmness and thixotropy. The firmness is measured by the resistance to a cutting wire produced by a standard cube of butter. Changes of body are correlated with changes in flavour as judged subjectively. Microphotographs of the fat structure are given both in normal and polarized light.

Weckel (148) describes a number of empirical rheological tests on butter: (*a*) the force required to crush a standard cube of butter, (*b*) the resistance to slicing with a standard spatula, (*c*) the time taken for a 'beam' of butter supported at the ends to sag by a given amount. A bibliography of such methods is given and the monthly variations in these properties are studied in several creameries. Methods (*b*) and (*c*) are regarded as of considerable practical value.

(*f*) Miscellaneous

Scott Blair *et al.* (149) have studied the chemical constitution and physical properties of bovine cervical mucus. A number of these properties are found to show characteristic values when the animal is in or near oestrus, noticeably the flow-elasticity, i.e. the extent to which a column of secretion, being expressed from a narrow tube by air pressure, retracts when the pressure is suddenly released. A simple apparatus (Oestroscope) is described in which a sample of secretion may be tested in the cowshed in a very short space of time. In this way it can be ascertained when cows are in season, or nearly so, at times when the usual behavioural signs are lacking or are not marked.

VII. ANALYSIS OF MILK AND DAIRY PRODUCTS

(*a*) Milk

Fat

Full instructions, based on much experience, for the sampling, preserving and testing of milk for fat content by the Babcock method, have been given by Wilster & Robichaux (150). Some of the factors concerned in the accuracy of the Babcock test have been the subject of other papers (151, 152, 153). Hautog (154), using several different types of butyrometers, has compared

the results obtained for the fat content of skim milk with the results obtained by the Röse-Gottlieb method. Schwarz *et al.* (155) have found Stigen's (156) modified Röse-Gottlieb method for the determination of fat in skim milk to be very satisfactory. They give a full description of the method; it saves time and reagents.

Gould (157) has investigated the influence of rancidity in milk upon the accuracy of the fat determination by the Mojonnier method. In milk in which, as a result of homogenization and storage, the extent of lipolysis was much greater than would normally be encountered, the analytical results were very slightly low. Starr & Herrington (158) made Mojonnier tests of butterfat containing a mixture of fatty acids resembling hydrolysed butterfat and found that only about one-quarter of the fatty acid mixture had been extracted. When single fatty acids were added to butterfat the proportion of acid extracted increased with increasing molecular weight of the acid. The lower fatty acids were appreciably volatile under the conditions of drying used in the Mojonnier test.

Lactose

A valuable paper on the various procedures in use for the determination of lactose in milk has been contributed by McDowell (159). The values for the lactose content of milk determined by the direct volumetric copper reduction method applied to unclarified milk and to clarified decalcified milk, by the polarimetric and iodometric methods applied to zinc hydroxide or cadmium hydroxide filtrates, and by the chloramine-T method applied to dialysed iron, phosphotungstic acid, cadmium hydroxide or zinc hydroxide filtrates, showed close agreement and may therefore be accepted as the true values. Clarification with acid mercuric nitrate or phosphotungstic acid for the polarimetric method, and with dialysed iron or phosphotungstic acid for the iodometric method, gave high results owing to failure to remove interfering substances.

For the rapid determination of the lactose content of samples of cow's and buffalo's milk, Ghosh & Datta Roy (160) diluted 10 ml. of milk to about 35 ml., added 5 ml. of 1% acetic acid and 5 ml. of dialysed iron, diluted to 100 ml., and filtered. The filtrate was titrated into Fehling's solution by Lane and Eynon's method.

Garrison (161) has pointed out that the A.O.A.C. official polarimetric method for the determination of lactose gives high results owing to the volume (2·6 ml.) allowed for the precipitated protein and fat being much too small. Sharp & Doob (162) have evolved a method for the quantitative determination of α- and β-lactose separately.

Minerals

Sanders (163) has reviewed at length the methods that have been employed for the determination of chloride in milk and has proposed a rapid modification of the accepted open Carius wet digestion Volhard titration procedure. One combined reagent containing silver nitrate, nitric acid and ferric alum is added to the milk and the mixture is titrated with potassium thiocyanate, the usual boiling being omitted.

For the determination of soluble calcium and phosphorus in milk, Pyne (164) has investigated the use of sera prepared by dialysis through cellophane, addition of rennet, addition of papain, and addition of mercuric chloride-borax reagent. As would be expected the results varied with the method. Tompsett (165) has described a method for the determination of lead in biological materials including milk. The complexes of copper, iron and lead are extracted from a solution of milk ash with sodium diethyldithiocarbamate, and the lead is separated and determined colorimetrically with dithizone.

Citric acid

The volumetric determination of citric acid by photochemical precipitation of acetone-dicarboxylic acid has been applied to phosphotungstic acid filtrates from milk by Fiala (166). Following Taufel & Schoierer's procedure (167) the citric acid is oxidized under the action of light, with iron and manganese salts as catalysts, to acetone-dicarboxylic acid which in the presence of Denigé's reagent forms an insoluble complex with mercuric sulphate. The mercuric sulphate content of the reagent and of the filtrate after removal of the complex is determined

by titration with sodium chloride and mercuric nitrate. A factor is given for the calculation of the citric acid from the reduction in mercuric sulphate observed. Recoveries of citric acid added to milk were satisfactory.

The recovery of citric acid by the usual modifications of the pentabromoacetone method is less than the theoretical value. Deysher & Holm (168) have shown that the loss of citric acid may be due to incomplete conversion of the acid due to too short a period of reaction, the volatility of the pentabromoacetone during drying, and its solubility in the reaction mixture and wash water. A standardized procedure is recommended which gave results within ±0·50 and ±1·0% of the theoretical values when applied to pure citric acid solutions and decitrated milk plus citric acid respectively.

Miscellaneous

Heinemann (169) has adapted for use with milk the well-known method of Mendel and Goldscheider for the determination of lactic acid in blood. The fat, protein and lactose are precipitated with copper sulphate and lime, the lactic acid is converted, by heating with sulphuric acid, into acetaldehyde and carbon monoxide, and the aldehyde is estimated from the depth of the red colour developed with veratrole (pyrocatechol dimethyl ester). The test described is accurate to ±0·004% in the range of 0·005–0·12% of lactic acid.

An interesting method for the determination of dissolved oxygen in milk has been devised by Sharp *et al.* (170) based on the complete reaction of the oxygen with reduced ascorbic acid in 5–15 min. at 25° C., in the presence of cucumber ascorbic acid oxidase. Ascorbic acid is added to the milk and determined by titration with 2:6-dichlorophenolindophenol before and after treatment with the oxidase. One atom of oxygen reacts with one molecule of ascorbic acid. Evidence of the reliability of the method is presented and data are given illustrating the use of the method to check the efficiency of a commercial milk de-aerator.

Menefee & Overman (171) have described a semi-micro-Kjeldahl method for the determination of total nitrogen in milk. About 1 g. of milk is digested with 2 g. of sodium sulphate, 5 ml. of sulphuric acid and 0·14 g. of mercuric oxide in a 300 ml. flask, and the ammonia is steam-distilled direct from the flask into 0·02*N* sulphuric acid or boric acid solution. Hunt (172) has used the indigo-carmine reaction as a test for chlorate and therefore indirectly for commercial hypochlorites (as these contain chlorate) in milk. Under the conditions of the test normal milk will decolorize the dye in 1½ hr.; milk containing 0·5 p.p.m. or more of chlorate will decolorize it in 15 min. or less.

A simple method for the approximate estimation of the *p*H value of milk and whey during cheese-making has been described by Davis & Thiel (173). A 1 in 20 dilution of milk or whey is treated with bromothymol blue or B.D.H. indicator 4460 and the colours are matched with a Lovibond comparator using appropriate disks graduated in 0·1 *p*H unit. Seekles (174) has described a rapid method for the same purpose using coloured 'Lyphan' indicator papers.

Ryan & Pyne (175) have found that their molecular constant for the detection of added water in milk can be determined, by making slight modifications, for milk which has been preserved with potassium dichromate for periods up to 2 months. Ludington & Bird (176) have found that the total solids content of skim milk can be estimated approximately by the use of the refractometer. With whole milk and cream the presence of fat causes difficulties.

Tables and monographs for reading the total solid content of milk from its fat content and specific gravity according to Babcock's (177) and Sharp & Hart's (178) formulae have been constructed by Lampert. Hutchinson (179) has extended the existing tables for the correction of the specific gravity of milk from the temperature of observation to the standard temperature of 60° F. to cover the range 40–90° F. Previously data were not available for temperatures above 80° F., and in tropical countries it is convenient to work above this temperature.

(*b*) Butter and butterfat

A new procedure termed the filtration method has been devised by Moir & Andrews (180) for the estimation of the iron and copper in butter. It has a considerable advantage over existing procedures in speed and simplicity of manipulation, and should be particularly suited to the

examination of large numbers of butter samples for control purposes. The method consists of the extraction of the metals from the curd into the aqueous layer of the melted butter sample, followed by the precipitation of the protein, and the colorimetric estimation of the metal in the filtrate.

A semi-micro method for the determination of the Reichert, Polenske, and Kirschner values of butterfat has been evolved by Dyer *et al.* (181). It deals with 1 g. of fat: approximate results are obtainable with 0·25 g. Approximately one-fifth of the usual quantities of reagents is used and the apparatus is reduced in size accordingly. 21 ml. of distillate are collected in 5–7 min., and of this 20 ml. are titrated with 0·02 *N* baryta solution. When compared with the results obtained by the standard method, which requires 5 g. of fat, Reichert values showed good agreement, Polenske values were a little low, and Kirschner values rather high.

Ogg *et al.* (182) have described a method for the determination of the salt content of an approximately 0·2 mg. sample of butter. The sample is picked from the mass of butter with a dissecting needle under a binocular microscope and transferred to a platinum microspoon. It is then weighed and ashed, and the salt is titrated with 0·01 *N* silver nitrate solution. The method is useful for the study of the distribution of salt in butter.

(*c*) Cheese

Full experimental details of the development and use of their extraction method for the determination of the volatile acids of cheese have now been given by Hiscox *et al.* (183). The paper also includes the results of the application of the method to several varieties of mature cheese. On the basis of the distribution of volatile acids, the nitrogen partition, and the *p*H, distinctions could be drawn not only between the white and blue-veined varieties as a whole, but also between the Roquefort and Stilton types of the latter varieties. In 1940 Marquardt & Dahlberg (184) drew attention to the advantage of accurate determinations of the volatile acids of cheese and suggested the use of a method similar in principle to the above extraction method. Later, however, they (185) discarded the steam distillation of pressure extracts and water-ether extracts of cheese in favour of a slight modification of the well-known direct steam distillation of a cheese mush.

Schloemer & Langmann (186) and Schwarz & Hagemann (187) have shown that sheep's milk cheese and cow's milk cheese may be distinguished by an examination of the extracted fats. The fat of sheep's milk cheese has a higher content of volatile acids, particularly of the water-insoluble fraction, and is pale green in colour with a bluish fluorescence under ultra-violet light. Wilster *et al.* (188) have given details of the determination of fat, moisture and salt in soft cheese.

(*d*) Miscellaneous

Continuing their researches on the factors affecting the solubility of milk powders, Howat *et al.* (189) have investigated the influence of speed and duration of stirring on the apparent solubility. Their results show clearly that for any given temperature the solubility cannot be regarded as an absolute value depending solely on the individual properties of the powder. Provided sufficient time is allowed for equilibrium to be reached, a different solubility level will be obtained for each speed of stirring employed. These findings are of considerable practical importance in connexion with the formulation of standard methods for determining the solubility of milk powders. Howat *et al.* conclude from their data that 30 sec. shaking of milk powder reconstituted in a 10% mixture is sufficient to dissolve its truly soluble portion, but that the protein which has become denatured during the drying process tends to pass into solution with increasing speed and duration of stirring. Under these circumstances it would appear that in devising standard methods for solubility determination a technique which involves a minimum of stirring or agitation is desirable. In the latter part of the paper a rapid method for determining the solubility of milk powders on a total solids basis, involving shaking for only 1 min., is described and discussed. This rapid method is also compared with the sediment test commonly used in industry.

Hankinson & Anderson (190) have described a new method for the detection of neutralized

cream based on the measurement of the *p*H and the buffering power of a serum prepared under standard conditions by the addition of uranyl nitrate to remove proteins, fat, and phosphates. The principal that the formation of lactic acid results in an increase in the buffering power of the serum, and the subsequent addition of neutralizer in an increase in *p*H, is used to reveal the presence of neutralizer. The method detected cream in which as little as 0·025% of lactic acid had been neutralized. A detailed comparison of the new method with a number of present methods from the point of view of accuracy and time required showed the new method to be the most suitable for the routine examination of cream samples.

Hillig (191) has reported that he found the Tillmans-Bohrmann method for the determination of the alkalinity of the ash of dried milk to give a satisfactory indication of the addition of neutralizer. He also reported on a study of methods for the determination of lactic acid in dried milk (192), and has given (193) a useful series of data illustrating the significance of the determination of the lactic acid content and the alkalinity of the ash of dried skim milk as an indication of the quality and possible neutralization of the initial fluid skim milk. The same paper gives full details of the author's colorimetric method for the determination of lactic acid, based on the extraction of the acid with ether and the production of a colour with ferric chloride. McVey & McMillin (194) have given details of methods for the detection and estimation of dried skim milk in sausages and similar comminuted meat products based on the determination of lactose and calcium.

Swope (195) has given full details of the Pennsylvania method, using standard Babcock equipment and an ammonia-butyl alcohol-sulphuric acid reagent, for the determination of the fat content of ice cream, ice cream mixes, sweetened condensed and evaporated milk, dried whole milk, milk, cream, buttermilk, and cheese. The results agree well with those of the Mojonnier method. Harmon & Renner (196) have described a simple vacuum total solids tester which can be assembled from ordinary laboratory equipment. Menefee & Overmann (197) have deduced equations which permit the calculation of the approximate total solids content of evaporated and condensed milk from the refractive index of their copper sulphate serum.

Cox & Wiley (198) have studied Pien, Baisse and Martin's rapid method for the estimation of diacetyl in dairy products colorimetrically with *p*-diaminobenzidine, and found that with modifications it gives results which compare adequately with those obtained by the usual tedious nickel dimethyl-glyoxime method. Schroeder & Racicot (199) have described a method for the detection of sodium alginate in dairy products, and England & Yongue (200) for the detection of annatto in cream.

S. J. ROWLAND
G. W. SCOTT BLAIR

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING
UNIVERSITY OF READING

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(*MS. received for publication* 14 *July* 1942)